University of Alberta

A DESCRIPTION OF AND INVESTIGATION INTO THE BIOLOGY OF *Ascogregarina glabra* N. SP. (APICOMPLEXA: GREGARINIDA: LECUDINIDAE) FROM *Gerris buenoi* (HEMIPTERA: GERRIDAE)

by

Rosalind Barrington Leigh

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**.

Department of Department of Biological Sciences

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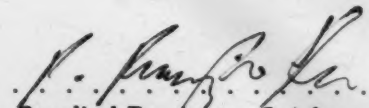

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Abstract

A new gregarine, *Ascogregarina glabra* n. sp. is described from the alimentary canal of all stages of *Gerris buenoi* (Hemiptera: Gerridae) and placed in the family Lecudinidae Kamm (Apicomplexa: Eugregarinida). This gregarine was also collected from *G. pingreensis*, and *G. comatus* in central Alberta, Canada, but was most prevalent in populations of *G. buenoi*,

Gametocysts developed and released oocysts within the host's alimentary canal, however, autoinfection did not occur. This gregarine apparently is not transmitted vertically, nor is it transmitted directly through the water column or by direct contact with conspecifics. Evidence is presented that suggests *A. glabra* is transmitted through an aquatic prey item transporting infective oocysts.

No pathogenic or beneficial effects of *A. glabra* were detected on *G. buenoi* adult size, juvenile development time or fecundity. A weak trend suggests that high gregarine loads inhibit initiation of flight in males, however further investigation is required to confirm this. Within the scope of this study, *A. glabra* is a benign symbiont and is not likely an important selective agent acting on its primary water strider host, *G. buenoi*.

To my mother, Iris, who has turned over stones and gazed into ponds with me since the beginning of time.

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Chapter 1

Introduction

1.1 Gregarines

The class Gregarinia is the most primitive and largest group in the phylum Apicomplexa with 1624 species, 231 genera, 40 families (Levine, 1988). Gregarines colonize the alimentary canals and body cavities of invertebrates, most commonly arthropods and annelids (Tanada and Kaya 1993).

Most eugregarines (Eugregarinorida) closely follow a generalised life cycle (Tanada and Kaya, 1993) but lacks asexual reproductive stage, or merogeny, which occurs in the related neogregarines. The life cycle includies five distinct life stages: oocyst, sporozoite, trophozoite, gamont, gametocyst (all terminology as per Levine, [1971]). The infective oocyst is ingested by the host. Once in the gut environment oocysts are triggered to release sporozoites, which emerge and attach to, or enter epithelial gut cells. Trophozoites absorb nutrients from the lumen, or from the host cell until either the host cell is sloughed from the epithelium, or the trophozoite is fully-grown and removes its mucron from the cell to become a gamont. Two of these mature gamonts undergo syzygy within the host to form a gametocyst with a protective hyaline coat within which oocysts form. Male and female gametes are released and combine to form zygotes, which then secrete a thick membrane to form an oocyst (Tanada and Kaya, 1993). The zygotes undergo meiosis and then mitosis to form (usually 8) haploid sporozoites within the walls of the oocyst. When the gametocyst is ready to dehisce, a radical change in the permeability of the outer gametocyst wall permits a large amount of liquid to enter. This increases the pressure within the gametocyst rupturing it at the weakest point to release oocysts. In some families oocysts are released in long chains through special pores (sporoducts); in other families oocysts emerge through tears in the gametocyst wall.

Three of the above gregarine stages live within the host (sporozoite, trophozoite

and gamont). The gregarine is expelled into the environment, usually as gametocysts (but sometimes as oocysts), which develop and release the infective oocyst stage.

Gregarines generally tolerate a narrow range of host species; in fact many species are restricted to one host genus (Levine, 1985). There are also reports of gregarines being specific to one species of host and even one insect host stadium (Åbro, 1976; Clopton et al., 1992). However, the generalisation that gregarines are mostly stenoxenous is largely derived from circumstantial evidence rather than experimentation. Cross-infection experiments between sister host species show that gregarines may infect a number of closely related species but do show differential infection rates and differential parasite development (Clopton and Gold, 1996; Lien and Levine 1980; Munstermann and Wesson, 1990).

Some gregarines may use a second species to act as a carrier of the infective oocyst. Only one such case has been verified (Åbro, 1976) but this is a possible transmission route, particularly for predacious hosts. In these cases, the gregarine is still considered to be homoxenous, using only one host, as it does not develop in or parasitise the carrier.

The mode of transmission of infective gregarine oocysts has been described for several groups of insects, in which the host ingests the infective oocyst directly from its environment (Beier and Harris, 1983; Clopton and Gold, 1996). Predatory hosts ingesting oocysts along with their prey (Åbro, 1976) and vertically transmitted gregarines (Wu and Tesh, 1989) are scarce in the literature.

The pathology of eugregarines is generally much weaker than for neogregarines, however, eugregarines can cause death in some cases, though this usually occurs in non-target hosts (Walsh and Olson, 1976) or in those already weakened (Dunkel and Bousch, 1968). The primary insult occurs through the destruction of epithelial tissues, blockage of food passage and interference with absorption (Åbro, 1971; Harry, 1967; Lipa, 1967), although some gregarines also breach the alimentary canal and expose the host to lethal bacterial infections (Tanada and Kaya, 1993).

1.2 *Gerris buenoi* Kirkaldy

Gerris buenoi Kirkaldy (Hemiptera: Gerridae) is a common waterstrider with a large range in North America (Andersen, 1993). This species is a habitat generalist

but occurs most frequently on temporary ponds (Spence and Scudder, 1980). *G. beunoi* adults are strong fliers, and make significant prereproductive and prediapause migrations (Spence, 1981). Like other gerrids, *G. beunoi* individuals are predator-scavengers that feed on a wide range of aquatic prey, emerging insects, conspecifics and terrestrial insects caught in the water tension (Rowe and Scudder, 1988; Spence and Andersen, 1994).

G. buenoi is partially bivoltine in central Alberta, meaning that some individuals complete juvenile development and breed directly in the same season. Overwintering adults are exclusively winged and emerge from diapause in the spring as the spring breeding generation. These gerrids then produce the second or "summer" generation, most individuals of which reproduce only after diapause (Spence, 1989). A small percentage of these summer generation adults breed directly and die before winter; some of these are apterous (Spence, 1989).

1.3 Protozoan parasites of Gerridae

Gerrids harbour several protozoan parasites. Trypanosomatids (Zoomastigina: Trypanosomatidae) are highly prevalent in populations studied around the world (Patton, 1908; Porter, 1909, Becker, 1923; Tieszen and Molyneux, 1989; Wallace, 1966). Arnqvist and Mäki (1990) found that trypanosomes reduced the overall vigour of *Gerris* adults but increased mortality only in food stressed hosts. Trypanosome prevalence is drastically lower in *Gerris* spp. populations in central Alberta, Canada (Klingenberg et al., 1997). However, there is a unique report of an as yet undescribed gregarine, which reaches high prevalences, but is apparently non-pathogenic to juvenile development of the host (Klingenberg et al., 1997).

1.4 Thesis objectives

Gregarines are an extremely rich and diverse group, which colonize a broad taxonomic and ecological range of hosts, and most of which remain undescribed or undiscovered (Levine, 1988). Before one can ask interesting evolutionary questions about gregarines much descriptive work must be done. In this thesis I provide the basic descriptive work for an undescribed gregarine inhabiting the alimentary canal of gerrids

in western Canada and probe selected aspects of the host-gregarine relationship.

A description of the gregarine life cycle and of each life stage is necessary for taxonomic identification, but also to understand the role of the symbiont within the host. These points, as well as the timing of the gregarine life cycle are addressed in chapter two.

The transmission mechanism of this obligate symbiont is critical in understanding the full life cycle of a gregarine species, but even more so in grasping how biotic and abiotic forces might impact the host-symbiont system. Chapter three addresses the development and release of the infective oocyst stage, and experimentally considers vertical, horizontal, and autoinfection.

In chapter four the pathogenic effects of the gregarine on *G. buenoi* are addressed by examining infected alimentary tracts, and statistically, by measuring the host growth and fecundity and the ability of individuals to take flight.

The final chapter is a summary and discussion of my important findings. In addition, I recommend directions for further research into this host-symbiont system.

1.5 Statement of Interest

G. buenoi were first examined for parasites to explain a growth pattern that contradicted the established life history theory, that there is a trade-off between development time and adult size (Roff, 1992). Klingenberg and Spence (1997) found a negative, instead of a postive correlation between development time and adult size. A pathogenic parasite may explain these findings by increasing the development time and suppressing the adult size in highly infected individuals.

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Chapter 2

Ascogregarina glabra n. sp. (Apicomplexa: Gregarinida: Lecudinidae) from the alimentary canal of three species of *Gerris* Kirkaldy (Hemiptera: Gerridae)

2.1 SYNOPSIS

A new aseptate eugregarine *Ascogregarina glabra* n. sp. (Apicomplexa: Eugregarinida) is described from the alimentary canal of all stages of *Gerris buenoi* (Hemiptera: Gerridae) collected in central Alberta, Canada. The gregarine was also found in *G. pingreensis* and *G. comatus* but not in co-occurring populations of the gerrid *Limnoporus dissortis* or the mesovelid *Mesovelia multisanti* (Hemiptera: Mesoveliidae).

The gregarine is placed as a new species in the Family Lecudinidae Kamm, 1922, Genus *Ascogregarina* Ward, Levine, and Craig, 1982. Trophozoites with small mucron, embedded into midgut epithelial cells. Gamonts solitary, loose in gut lumen; length 889.0 ± 369.15 μm and width 89.3 ± 46.47μm. Gamont nucleus spherical with no fixed location, with visible nucleosis. Association of gamonts not observed, assumed very late. Gametocysts spherical; diameter 383.8 ± 54.52 μm; hyaline coat approximately 20 μm in mature gametocysts. Gametocysts dehisce by simple rupture. Oocysts irregularly shaped; diameter 10.7 ± 1.02 μm. Evidence supporting *in vivo* sporulation, but not autotransmission within the host is presented. The gregarine takes approximately 16-20 days to develop within its host.

2.2 INTRODUCTION

There is a large amount of variation and diversity within the Class Gregarinia. The taxonomy is presently based on measurements and morphology of the different stages, the mode of oocyst dehiscence, and host taxonomy (Clopton and Lucarotti, 1997). Some uncertainty has been shed upon the "species" designation of gregarines, as there is evidence that different strains of gregarines differentially infect hosts (Clopton and Gold, 1996). These problems will likely require a genetic approach to make final discriminations between species (McManus and Bowles, 1996).

Gregarines infecting the family Gerridae (Hemiptera) have never been discussed. Poisson (1957) first reported them in passing from a European *Gerris* sp. and Klingenberg et al. (1997) first reported gregarines in North America (Alberta, Canada) from *G. buenoi* at our study site, however it has not yet been described.

In this work I describe and document the life stages and the life cycle of the gregarine (Apicomplexa: Gregarinida) infecting *Gerris buenoi* Kirkaldy (Heteroptera: Gerridae) in Alberta, Canada.

2.3 METHODS

2.3.1 Dissections of potential hosts

Gregarines were obtained from dissections of *Gerris* spp. hosts collected from Meadow pond and George Lake, at the George Lake field site (114° 06' W, 53° 57' N, 90 km NW of Edmonton, Alberta). The gerrids were stored alive at approximately 4°C until dissected.

For dissection, the insects were decapitated thereby largely removing the foregut from the examination. The specimen was placed ventral side up and cut from the first thoracic segment to the 7^{th} abdominal segment. The alimentary canal was immersed in Ringer's solution by filling the entire haemocoelic cavity. The entire remaining length of the alimentary canal was removed and placed on a slide in a drop of Ringer's solution for examination under a dissecting microscope (Wild M5). The alimentary canal was then opened up along its entire length, covered with a cover slip and examined under a compound microscope (Leitz).

In initial dissections (n=50), the gonads, fat tissue and alimentary canal were

examined under a dissecting and compound microscope for evidence of parasitisation. In subsequent dissections all tissues were examined under a dissecting scope; only alimentary tissues were examined under the compound light microscope.

2.3.2 Host Range

Samples (n=50) of all *Gerris* spp. found in Alberta (*G. buenoi*, *G. pingreensis*, *G. comatus*) as well as *Limnoporous dissortis* Drake and Harris and the mesovelid, *Mesovelia multisanti* (Hemiptera: Mesoveliidae) were collected from Meadow Pond and/or George Lake. These samples were examined for gregarines.

2.3.3 Gregarines

All stages of the gregarine life cycle were examined and measured using a compound light microscope (Leitz) fitted with an eyepiece micrometer. Gamonts, trophozoites, gametocysts and crescentic structures were present in the host gut and measured directly: gamonts (length, width), trophozoites (length, width), gametocysts (the longest and shortest axes α, β) and crescentic structures (length).

To obtain gametocysts from gerrid, adult *G. buenoi* were placed in small petri-dishes on black paper and left for 24 hours.

To obtain oocysts and sporozoites, gametocysts were removed from the host gut and submerged in Ringer's mixed with fluid from the alimentary canal of the host (approximately 1:1) on well slides. The well slides were placed inside moist chambers consisting of petri dishes lined with saturated tissues to maintain a high humidity. The gametocysts were allowed to mature, releasing oocysts and then sporozoites for examination.

All preparations were photographed in colour on a Polyvar differential interference contrast microscope. Images were later converted to black and white prints for publication.

2.3.4 Estimations of Timing of Gregarine infections

Samples of instars 2-5 of *G. buenoi* were collected from Meadow Pond on August 9, 1994 when the gregarine prevalence in the adult population was close to 100%. The earliest appearance of 4 different gregarine stages (trophozoite: small and medium,

gamont, gametocyst) was recorded. Assuming an average of 5 days between *G. buenoi* moults under field conditions at this time (unpublished data), and instantaneous transmission of infective oocysts to new hosts, the rate of gregarine development was estimated.

2.4 RESULTS

2.4.1 Gregarine host and tissue range

All stages of a gregarine, diagnosed as belonging to the genus *Ascogregarina*, were found in *G. buenoi*, *G. comatus* and *G. pingreensis*. No evidence of gregarine infection was found in either *Limnoporous dissortis* or *Mesovelia multisanti*. Gregarines were present in all *G. buenoi* examined but prevalence was much lower in the other *Gerris* spp. (Table 2.1).

Gregarines appeared exclusively in the alimentary canal attached directly to epithelial cells and extending into the gut lumen; the family Gerridae does not develop a periotrophic membrane (Werner et al., 1991). There was no evidence of parasitisation in the reproductive organs, haemocoel or fat tissue.

2.4.2 Description of gregarine stages

Note: Please refer to Table 2.2 for measurements of all gregarine stages.

Trophozoites

Young trophozoites are broadly oval and attached directly to the gut epithelium with a rounded mucron (Figure 2.2a-c). No intracellular stage was observed. As the trophozoite develops it becomes more elongate with a tapered posterior end resembling the gamont (Figure 2.2c). The trophozoites retain a much larger length:width ratio than the gamont.

Trophozoites attach to host epithelium throughout the midgut (first, second, third ventricles), but are most abundant in the first ventricle (Figure 2.1). When trophozoites are disturbed during dissection the larger ones frequently detach from the host epithelium. The host cell is ruptured in this process and a part of the host epithelial cell remains attached to the mucron to form a "hood".

Gamonts

Gamonts are aseptate, straight and elongate. The mucron is retained by the gamont but is much reduced from that of the trophozoite. The gamont mucron is much smaller and knob-like, with a rounded end (Figure 2.2d). The gamont is long and narrow, tapering to a fairly sharp point at the posterior end. The cytoplasm has a coarse granular appearance. The gamont nucleus is spherical and appears at any position within these organisms. A large spherical nucleolus is visible in trophozoites and mature gamonts (Figure 2.2a, d). Gamonts are minimally contractile and move by a simple gliding motion.

Gamonts are without exception solitary; no bio-associative stage was observed. Gametogony does, however, occur as evidenced by the bi-lobed appearance of immature gametocysts. Syzygy was not observed once in over 2000 dissections, suggesting that syzygy is a very rapid transition from two individual gamonts to the formation of a gametocyst.

Gamonts appear in all regions of the midgut, but in largest numbers in the first ventricle. There is a severe constriction at the posterior end of the first ventricle that appears to prevent the gamonts from being pushed down the gut by peristalsis (Figure 2.1). As a result, large aggregates of gamonts are caught in the first ventricle. When in large numbers, they align longitudinally and completely fill and distend the first ventricle. Few individuals were found in the second ventricle and fewer still in the third, except in very severe infections.

Gametocysts

Gametocysts are large and spherical (ratio of longest and shortest axes is $0.98 \pm 0.07\mu$m). The gelatinous hyaline coat surrounding the gametocysts is very thin (approximately 20μm). Occasionally, the hyaline coat is bright red or pink. Material in the *G. buenoi* gut is often brightly coloured due to pigments found within its prey (personal observation); these observations suggest that some material used to construct the hyaline layer is taken directly from the gut lumen.

When first formed, gametocysts have a transparent sheath through which a clear division can be seen between the two gamonts (Figure 2.3a). As the gametocyst matures, this division disappears and a hyaline sheath is formed. The gametocyst appears white and opaque under reflected light and dark grey and granular under

transmitted light (Figure 2.3b). Occasionally, malformed gametocysts are observed; these cysts are very small and misshapen and probably the result of an unpaired gamont attempting to encyst (Hoshide and Todd 1993).

As the gametocyst nears rupture, an inner membrane withdraws from the outer hyaline sheath. Initially oocysts are released through one opening or polar plug (Figure 2.3c), however, once gametocysts are mature any disturbance, (e.g., gentle touch with a probe) causes them to rupture, discharging oocysts violently in all directions. This was observed in gametocysts matured in moist chambers as well as in samples taken directly from the host gut in dissection implying that the entire maturation process does occur within the host. In completely dehisced gametocysts, the hyaline sheath deflated and collapsed.

Gametocysts occur throughout the midgut and in the hindgut; gametocysts are most often clumped just before the sphincter between the first and second ventricle, or accumulate in the hindgut. Muscular peristalsis by the host gut might propel them out of the host, however, no gametocysts were found emerging from live hosts.

The gametocysts of these gregarines are extremely conspicuous in the gut. The gametocysts are larger in diameter than the second ventricle, so that when passing through this section, they distend the gut wall. Distension of the gut wall was also observed with large numbers of gametocysts in other parts of the midgut (Figure 2.1). Loads of up to 60 gametocysts were found within a single host.

Oocysts and Sporozoites

Oocysts were obtained from dehiscing gametocysts. Gametocysts and oocysts were observed to sink in both Ringer's solution and freshwater.

Oocysts are irregularly shaped, but have severe angles (Figure 2.3d). No oocysts were observed loose within the alimentary canal.

Groups of oocysts emerge from dehiscing gametocysts in "clumps" enshrouded in a gelatinous substance (Figure 2.3c). The oocysts are not attached to each other as they emerge from the gametocyst, as in sporulating gametocysts of some other gregarines. These clumps of oocysts are slightly "sticky" and adhere loosely to the bottom of the well slide. When oocysts dry out, they adhere to the substrate surface until re-hydrated (as observed by Hoshide and Todd, 1993).

Mature oocysts ready to release sporozoites "jump" around in a haphazard fashion. This is due to eight sporozoites moving independently within the oocyst case. Sporozoites emerge one at a time from ragged tears in the oocyst wall. Emerged sporozoites exhibit a vigorous thrashing motion (similar to that of nematodes whose movement is caused solely by longitudinal muscles). Sporozoites are long and slender and taper to a point at both ends.

Crescentic structures

Dissections of the host gut revealed crescent-shaped structures. The crescentic structures curved to approximately 45 degrees (Figure 2.3e). They are slightly thickened in the middle and tapered to a sharp point at either end. These structures appear completely inert both within the host and without.

The crescentic structures were regularly observed within the alimentary canal of hosts, in particular those with gregarine infections consisting of mature gametocysts. They appeared densely clustered in a gelatinous matrix, often at the junction of the first and second ventricle where the gut narrows to less than the diameter of a gametocyst (Figure 2.1).

The association of crescentic structures with gametocysts was observed after gametocysts were transferred from the host gut to glass slides. Gametocysts that burst during handling were examined under a compound microscope. Once a gametocyst was partially ruptured, the tightly woven network of crescentic structures embedded in the gelatinous layer was detectable. The same was observed in gametocysts that matured and released oocysts in well slides. Once oocysts were released onto the slide they remained embedded in a combination of dispersed gelatinous matrix and crescentic structures.

2.4.3 Estimations of timing of gregarine infections

Gregarines were found in all stages of the host *G. buenoi* (except eggs). In samples of first instar *G. buenoi*, the most advanced gregarine stage was a very small trophozoite attached to the midgut epithelium. These trophozoites ranged from the very early developmental stage which were almost spherical, to slightly elongated oval trophozoites. The second instars had larger trophozoites that were still attached to

the host cells. Gamonts (detached trophozoites) were first observed in third instar larvae. The fourth instars were the earliest larvae to harbour gametocysts. Fifth instar nymphs had all gregarine stages and the largest proportion of gametocysts.

Using an average time of 5 days (unpublished data) between *G. buenoi* moults in late August, the number of days required for the gregarine to reach each developmental stage can be estimated. All these estimates are minima as they are reflected by the gregarine's first appearance in this population: earliest infected host and fastest developing gregarines. The gametocysts would take approximately 16-20 days to form in the host. Adding 72 hours that it takes for gametocysts to develop, release oocysts and then release active sporozoites (unpublished data, see chapter 3), it would take an estimated 19-23 days for this gregarine to complete one full life cycle. This estimate does not include time required for transmission of mature oocysts to a new host, or autoinfection.

2.5 DISCUSSION

2.5.1 Taxonomical placement

This gregarine is placed in the Order Eugregarinorida Léger, 1900 as merogeny is absent, while gametogeny and sporogeny are present in the life cycle. The gamont is composed of a single compartment with no protomerite or deutomerite, placing it in the Sub Order Aseptatorina Chakravarty, 1960. This gregarine is placed in the Family Lecudinidae Kamm, 1922: gamonts are elongate, with forward gliding motion. It could not be placed in other families based on gamont motion, morphology of gamonts, gametocysts, and oocysts.

This gregarine is placed into the Genus *Ascogregarina* Ward, Levine, and Craig, 1982: simple mucron present, gamonts spatulate, gametocysts spherical, and occurs in an insect. All the species in this genus, except for one (found in Coleopterorida) are parasites of Dipterans, and predominantly mosquitoes (Levine, 1988). However, the gregarine here described from gerrids is not congruent with any of the specific descriptions within this genus, therefore a new species is proposed.

The species epithet *glabra*, meaning smooth or bald was chosen to describe this gregarine because it has very smooth lines, has little shape along its length, and has a very small and inconspicuous mucron lacking barbs or hooks.

2.5.2 Host specificity

Ascogregarina glabra n. sp. was restricted to the Genus *Gerris* in Alberta. The ascogregarine was able to complete syzygy in all three *Gerris* spp., however, a very high prevalence within *G. buenoi* contrasted low prevalences in the other *Gerris* spp. Therefore *G. buenoi* appears to be the primary host.

There is no description of the gregarine in Gerridae mentioned by Poisson (1957), therefore no comparison can be made.

Biological limitations that define a gregarine's host range can occur at several junctures in the gregarine life cycle. Some component of the host gut (pH, enzyme, or combination) triggers ingested oocysts to release active sporozoites once ingested by a receptive host (Tanada and Kaya, 1993). This response has been elicited in the laboratory by using homogenized host gut tissue (Hoshide et al., 1993; Clopton and Gold, 1996) and by using weakly acidic solutions of pepsin (Åbro, 1976).

The second host-parasite compatibility test is when the emerged sporozoites attempt to penetrate a host cell. *Gregarine blattarum* sporozoites emerged in gut homogenates of all examined cockroach species (5), but were only able to establish infections in one cockroach species (Clopton and Gold, 1996). Walsh and Olson (1976) found that *Lankesteria culicis* gregarines emerged within but could not penetrate the periotrophic membrane of some *Culex* spp. Finally, infections can establish within non-ideal hosts, but may exhibit lower prevalence within the host population, or be unable to complete their life cycle (e.g. gametogeny) (Lien and Levine, 1980).

2.5.3 Crescentic structures

Crescentic structures ("sickle shaped bodies") have been reported before from neogregarines, however, they were thought to be sporozoites released from oocysts, and therefore proof of autoinfection (Finlayson, 1950; criticism in Zizka, 1972). The crescentic structures presented in this study are uniquely reported from an eugregarine and are clearly not reproductive in nature. They are completely inactive in all media, and in the host gut, whereas sporozoites emerging from oocysts are extremely active. Furthermore, the crescentic structures are over three times the length of the oocysts, and therefore could not be packaged in groups of eight within one oocyst: thus I conclude that the crescentic structures are a structural component of the gametocyst

wall.

2.5.4 *Ascogregarina glabra* life cycle

The life cycle of *A. glabra* n. sp. requires approximately 16-20 days to develop with its host, and 19-23 days to complete its life cycle. These estimated rates may vary with environmental conditions, however, the life cycle is sufficiently rapid to allow many generations within one season. This enables the parasite to rapidly magnify its prevalence within the host population within one season. Furthermore, infections were found in first instar larvae (<5 days old) implying an extremely efficient transmission rate; this suggests that the earlier assumption of instantaneous transmission is not so unreasonable.

The gerrid provides a stable and continuous environment for this gregarine. The life history of some gregarine hosts necessitate coordination of gregarine development with that of the host (Beier and Craig, 1985). This is not the case for *A. glabra* as the gerrid hosts do not undergo holometably, both larvae and adults utilise the same habitat, and the length of the gregarine's reproductive cycle is short in comparison to that of its host (<0.5 times).

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Table 2.1 Gregarine Prevalence in Gerridae of Alberta, August 09, 1994.

	Prevalence (n)	Gamonts	Gametocysts
G. buenoi	1.00 (50)	+	+
G. comatus	0.07 (44)	+	+
G. pingreensis	0.16 (30)	+	+
L. dissortis	0.00 (30)	-	-

+ means present, - means absent

Table 2.2 Measurements of gregarine stages taken from *G. buenoi*

	Sample Size	Length (μm) Mean (S.D.)	Width (μm) Mean (S.D.)	Ratio (L:W) Mean (S.D.)
Trophozoites	n=183	404.1 (147.9)	117.3 (165.7)	8.4 (8.0)
Gamonts	n=159	889.0 (369.1)	89.3 (46.5)	10.7 (4.5)
Oocysts	n=10	10.7 (1.0)		
Crescents	n=10	34.7 (1.7)		
		α Axis (μm)	β Axis (μm)	Ratio (α:β)
Gametocysts	n=39	383.8 (54.52)	393.4 (63.97)	0.98 (0.070)

Figure 2.1 Alimentary canal of *Gerris buenoi*. Midgut is composed of three distinct ventricles (v1, v2, v3); constriction between v1, v2, c; Malpighian tubules, mt; Hindgut, hg; Rectum, r; Rounded edge of gregarine gametocyst (g) in v1.
40 X Magnification.

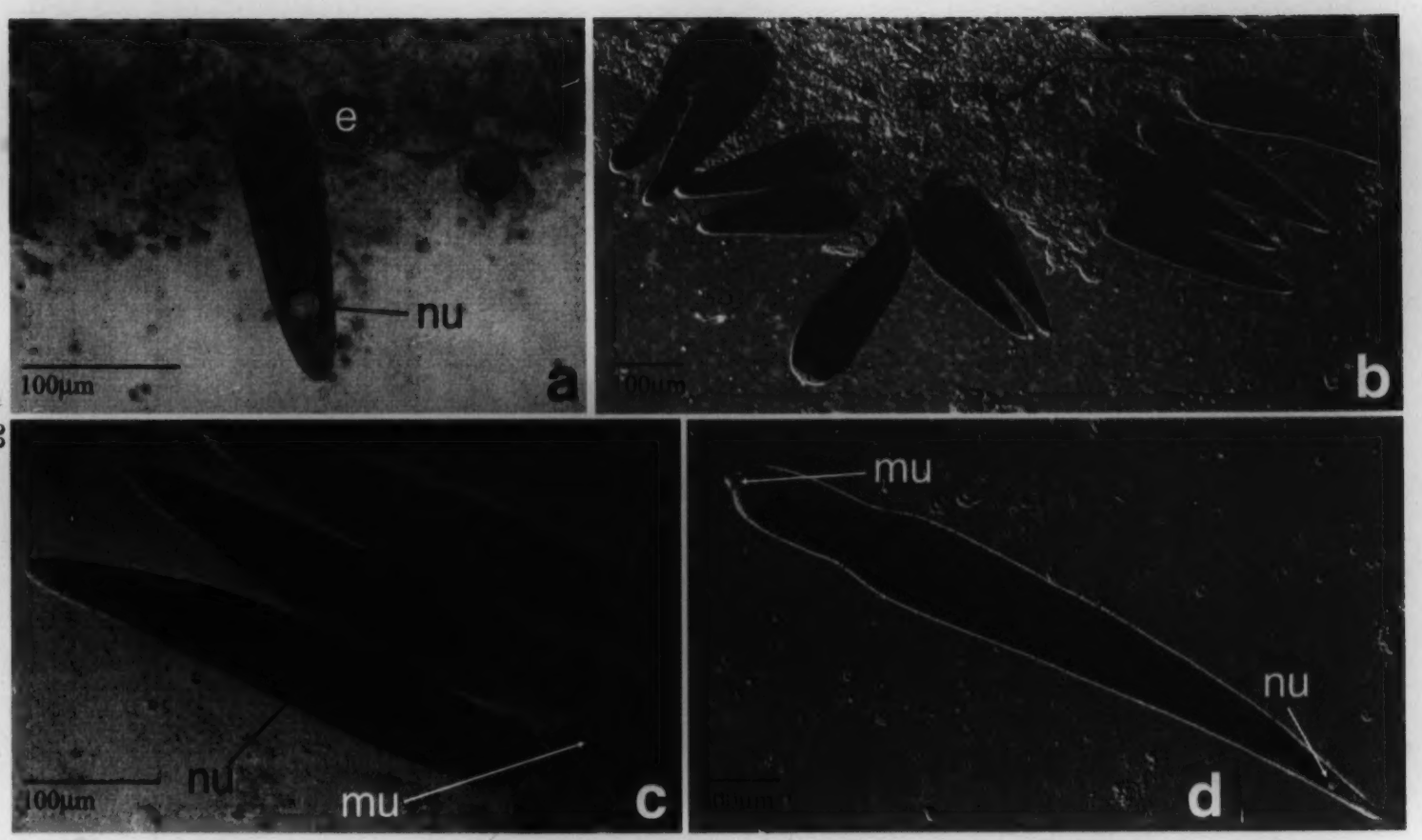

Figure 2.2 Gregarine stages: trophozoites, gamont. a) young trophozoite embedded in host epithelial tissue (e); nucleus, nu. b) Cluster of trophozoites. c) Larger trophozoites showing mucron (mu) embedded in epithelial tissue. d) Mature gamont in gut lumen with retained mucron

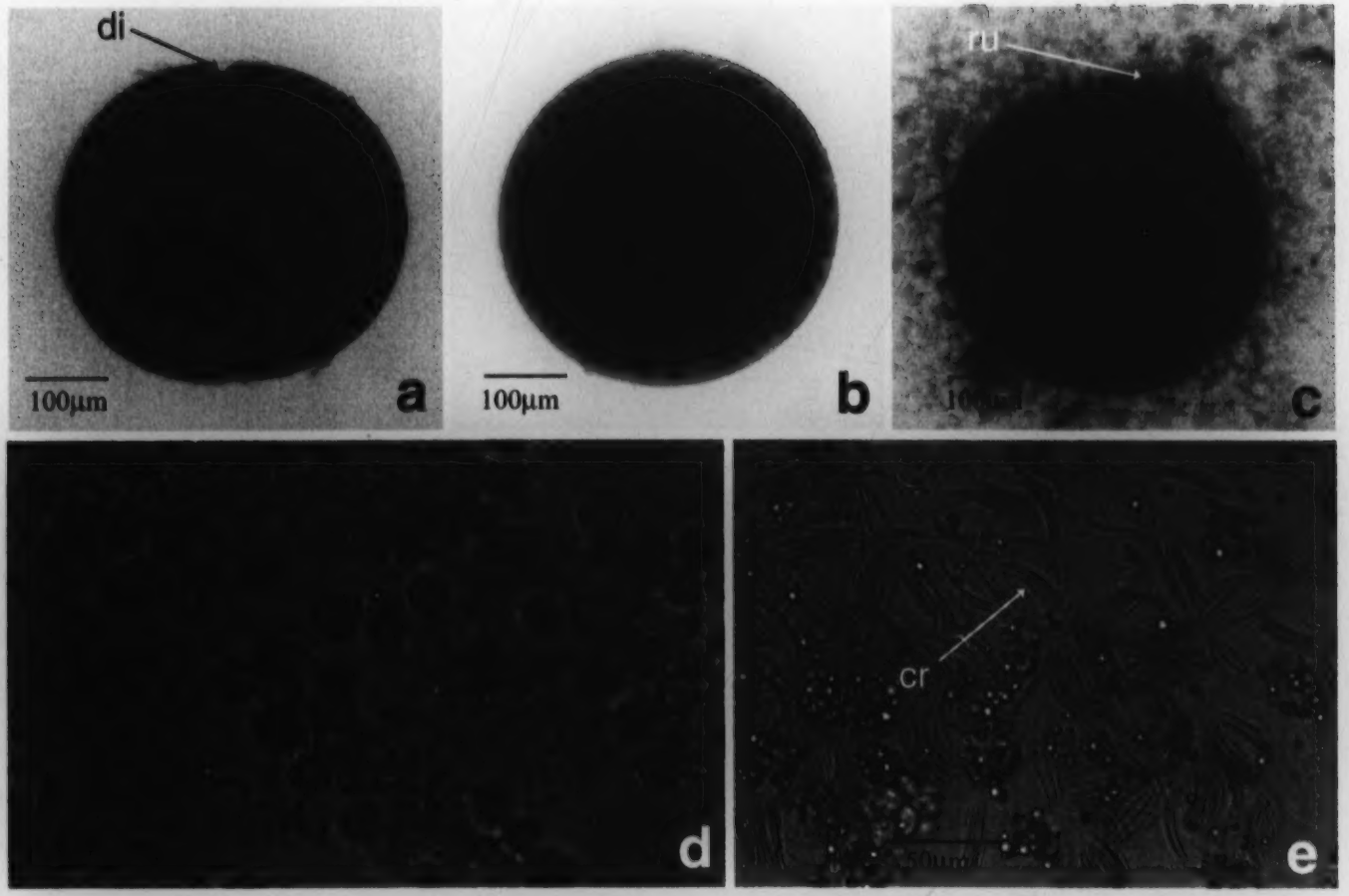

Figure 2.3 Gregarine stages: gametocyst, oocysts, crescents. a) Newly formed gametocyst with the two formative gamonts still distinguishable; division, di. b) Gametocyst fully formed. c) Gametocyst releasing oocysts from one ruptured (ru) point. d) Oocysts e) Cluster of crescents (cr) from degraded gameocyst wall

Chapter 3

Transmission and life cycle of *Ascogregarina glabra* (Apicomplexa: Gregarinida: Lecudinidae) from the alimentary canal of *Gerris* spp. (Hemiptera: Gerridae)

3.1 SYNOPSIS

The mode of transmission of the gregarine *Ascogregarina glabra* (Apicomplexa: Gregarinida: Lecudinidae) to its primary host *Gerris buenoi* (Heteroptera: Gerridae) is investigated.

Gametocysts were found to develop and release oocysts within the host's alimentary canal, however, autoinfection is unlikely and not supported in this study.

Experiments in both laboratory and field provide evidence that the gregarine infective stage (oocyst) is not transmitted to the host directly from the water column, from vegetation, direct and indirect contact with conspecifics, or through aerial prey. Gerrids on food limited regimes showed reduced growth and had larger gregarine infections under field conditions, as measured by the number of gametocysts in teneral adults.

Gregarine infection is subject to extremely strong spatial effects within one water body (Meadow Pond). This observation suggests that transmission is effected through an aquatic prey item transporting infective oocysts.

3.2 INTRODUCTION

The mode and resulting efficacy of a parasite's transmission have enormous implications in the evolution of host-parasite relationships. Host-parasite systems are often in terms of tradeoffs, the most commonly cited being between transmission and virulence of the parasite (Frank, 1996). Unlike free-living organisms, parasites are constrained by their external environment and additionally by the life history of their hosts. Therefore, many parasites, particularly those that are highly host-specific adapt very closely to the host's life history.

The mode of gregarine infection is well established for a number of gregarine-host relationships. There are no reports of transovarial vertical transmission in gregarines, although, there is a report of vertical transmission through surface-contaminated eggs (Wu and Tesh, 1989). Rather, almost all reports show direct infection through accidental ingestion of oocysts by the host. The concept is simple for scavenging or herbivorous hosts with chewing mouth parts and has been described in both terrestrial and aquatic hosts (Åbro, 1976; Beier and Harris, 1983; Beier and Craig, 1985). This has been verified in both terrestrial and aquatic infection regimes and subsequently emulated in the laboratory (Clopton et al., 1992; Beier and Craig, 1983; Clopton and Gold 1996).

The rate of successful development by the reproductive stage of a parasite is an important factor in transmission as it determines the number of infective stages at large (Mackinnon and Read, 1999). In gregarines, this stage is the gametocyst. Gametocyst development is sensitive to both humidity and temperature (Patil et al., 1983). Temperature optima for sporulation and dehiscence varies among species, but generally lie between 30-40 ° C (Patil et al., 1983). Different species are characterised also by different temperature ranges within which they can develop; reported minima range from 15 to 25°C (Åbro, 1990; Patil et al., 1983). Humidity requirements may be more flexible than temperature, as a 50% dehiscing success rate has been observed at all humidity levels except 0% and 100% (at which none dehisced) (Kolzoff, 1958). Exceptions to this occur in gregarine species in which gametocysts develop within the host and exit into the environment as oocysts. A clear understanding of the environmental limitations for parasite development are crucial for testing various hypotheses about transmission.

Transmission rate will ultimately depend on frequency of encounter between host

and infective gregarine stage (Janovy and Kutish, 1988); therefore, there is usually some mechanism at play to increase the local density of the infective stage (Keymer and Anderson, 1979). Three mechanisms are described in the literature. In terrestrial systems, hosts often excrete waste during or shortly after feeding resulting in high oocyst density at host feeding sites (Clopton et al., 1992; Clopton and Gold, 1996). Second, in aquatic systems, gregarine populations appear to prosper best in small volume water bodies where high oocyst concentrations are more easily maintained (Beier and Craig, 1985; Åbro, 1987). Third, oocysts can be transmitted to predatory hosts via their prey, which act as an oocyst concentrating mechanism; however, there are few well-established examples of this (Åbro, 1976). Infection regimes for gregarine-host systems involving predatory hosts are more difficult to resolve due to their complexity and the difficulty of oocyst detection in the evironment (Åbro, 1976). The life history of the gregarine host therefore beomes essential in piecing together the details of the gregarine life cycle.

Gerrids (Hemiptera: Gerridae) are hemimetabolous, and all stages use a similar ecological niche (Anderson, 1982) thereby providing a more-or-less continuous environment for parasites. However, gerrid life history presents several constraints for the transmission and development of this gregarine. The uptake of oocysts is constrained by their semi-aquatic life style and especially by the fact that gerrids feed through a narrow proboscis. Further, temperate gerrids undergo diapause as adults (Anderson, 1982; Spence, 1989) and thus a dormant period for the symbiont is required, either within or without the host. In this work, I investigate the transmission of the *Ascogregarina glabra* and its life cycle in relation to the life history details of both the gregarine and the pond skater *G. buenoi* Kirkaldy.

3.3 METHODS

3.3.1 Retrieving gametocysts

In order to retrieve gametocysts for use in later experiments, 30 adult gerrids were collected from Meadow Pond where gregarine prevalence was known to be high, and maintained in the laboratory in covered petridishes. Each petridish was placed on a piece of black paper and examined under a dissecting microscope for evacuated gametocysts daily, for two weeks.

3.3.2 Factors affecting gametocyst maturation

An experiment was run to assess whether or not gametocysts could complete the life cycle within the host, and therefore have the ability to autoinfect the host, and secondly to determine whether gametocysts mature faster in the host gut, or in a fresh water environment.

Thirty-five gametocysts were obtained through dissection from one individual gerrid. The infection in this individual appeared to be the result of one infection event: it consisted solely of gametocysts in which the two fusing gamonts were just discernible as separate entities within the cyst. The gametocysts used in this experiment all developed within one host and therefore under similar conditions (e.g. nutrient availability, general chemical environment).

Ten gametocysts were submerged in each of three (3) treatment media on well-slides placed inside moist chambers. The first treatment consisted of freshwater (from Meadow Pond) simulating gametocyst release directly into the pond. The second treatment was Ringer's solution and the third was Ringer's solution with host gut tissue added, simulating the host gut lumen. For treatments 1 and 2 the gametocysts were washed in their medium several times to remove any gut residue. All treatments were maintained at room temperature (17°C) and immersed in the respective medium for the remainder of the experiment.

In the process of setting up this experiment,(during the transfer from host gut to the treatment slide) all gametocysts were exposed to ambient oxygen which could be a signal to the parasite that it has left the host.

Every 24 hours (for 72 hours), each treatment slide was placed under a dissecting microscope and examined for release of oocysts. Once gametocysts began dehiscing, samples of the released oocysts were examined under a compound microscope for oocyst activity and the release of sporozoites. Results were recorded every 24 hours and development was classified into four stages: 1) Immature: cyst wall formed but two gamonts are still clearly visible, 2) Mature: cyst wall filled out, gamonts no longer visible; no evidence of cyst wall breach, 3) Dehiscing: cyst wall breached in at least one place and oocysts being released, 4) Oocysts active and releasing sporozoites.

3.3.3 Laboratory infection attempts with viable oocysts

Gametocysts from dissections of gerrid adults were placed in moist chambers until oocysts were released. Samples of oocysts were removed and exposed to host gut extract to test for viability (release of sporozoites); the remainder of the oocysts were placed in the water of live gerrid cultures.

Gerrid nymphs (instars 1-3) and adults were taken from laboratory cultures (not infected with gregarines) and placed in mass cultures (n=20) in shallow 1.8L containers (to maximise oocyst density). Gerrids were fed lab-raised flesh flies (*Sarcophaga bullata* Parker). Viable oocysts from five gametocysts were placed in the water of each culture, which was aerated with minimal disturbance to the water surface. Rough counts of oocysts give a conservative estimate of 500 oocysts per gametocyst giving a concentration of 1.4 oocysts per ml. It is unlikely that natural oocyst concentrations in open water could exceed that of this laboratory trial. The gerrids were left on the treated water for one week and then examined for gregarine infection.

3.3.4 Laboratory infection attempts by habitat variation

A pilot experiment attempting to infect gerrids in the laboratory was conducted. Uninfected (lab reared) first instar gerrids were individually reared under four different treatments in the laboratory. The control gerrids were held on tap water and fed laboratory-bred flies (*S. bullata).* Treatment gerrids were raised on pond water (Meadow pond), or with a leaf of *Potomogeton* sp. (from Meadow Pond), or were fed insects caught in a light trap (primarily consisting of green midges).

The background gregarine prevalence at the time of set up (July 30, 1994) was 0.86 in 2nd instars (n=28) and 0.92 in 3rd instars (n=38). Each gerrid was assessed for gregarine infection within 24 hours of its imaginal moult. At the approximate time of dissection (August 26, 1994) the background gregarine prevalence was 1.0 in fifth instars (n=24) and 0.88 in teneral adults (n=34).

3.3.5 Vertical transmission

To investigate the possibility of vertical transmission, the reproductive tracts of female adult gerrids were examined for evidence of oocysts. Further, ten laboratory hatched offspring from each of ten infected females were reared to the third instar

(approximately 15 days old) in the laboratory and then examined for gregarine infections.

3.3.6 Transmission in field-varied habitat structures

Two field experiments were run contemporaneously to test how gregarine infections varied with manipulations of host environmental structure. The experiments were run approximately 6m apart to reduce any interference between the experiments; each experiment included an independent control treatment.

In the first experiment, I used treatments designed to increase transmission based on a specific transmission hypothesis. The control gerrids were raised individually and fed laboratory-raised flies daily. Food limited gerrids, fed only every other day, tested the hypothesis that nutrient stress weakens the host making it more amenable to the initiation or maturation of a parasitic infection. Gerrids raised in groups of three (triplets) tested the hypothesis that gerrids transmit infections horizontally through direct (touching) and indirect (via frass or food sharing) physical contact between conspecifics. The vegetation, in which each gerrid was provided with one leaf of *Potomogeton* sp., tested the hypothesis that a resting site is important in parasite transmission (Porter, 1909) by keeping oocysts at the water surface for ingestion.

The experiment used laboratory-reared first instar summer generation gerrid larvae, which were set up within 24 hours of hatching in the laboratory. These were raised under the four different conditions in three adjacent enclosures on Meadow Pond (Figure 3.1). All four treatments were replicated in each enclosure, separated by compartment walls. Treatments were systematically allocated to maximise positional variation for each. At set up, each treatment was represented by 60 individuals, except in the triplet in which there were 180. Each gerrid was checked every day and fed *ad libitum* (except food limited treatment).

All gerrids were dissected and assessed for gregarine infection within 24 hours of their imaginal moult. Total gregarine load was calculated by adding the number of trophozoites, the number of gamonts, and twice the number of gametocysts (as each gametocyst is formed by two gamonts). Gametocyst load was calculated by doubling the number of gametocysts found in the gerrid; this parameter was used in the analyses as it reflects the long term infection rate: the number of gregarines that developed in the host over the previous 16-20 days, or one half to two thirds of the

larval development time (unpublished data).

The total gregarine load data and gametocyst load data were transformed (log[x+1]) to correct for a skewed distribution and then analysed using a general linear model (SAS, 1990 proc GLM for unbalanced data). This analysis was completed using both total gregarine load, and gametocyst load. Planned contrasts (linear comparisons) were computed for each treatment mean against the control mean.

The triplet treatment was tested to see if gregarine infections were randomly distributed between tubs, within treatment replications. A separate Chi-square test was run for each of the three triplet replicates (compartments). Expected values for the number of tubs having each of 0, 1, 2, and 3 infected gerrids were calculated using the gregarine prevalence of each replicate.

In experiment 2, a separate enclosure (Figure 3.1) was designed to detect gregarine transmission through the water column. In this experiment, infected (gregarine prevalence of 0.93-0.95) gerrids collected from Meadow Pond were used to locally increase the density of infective gregarines. Twenty first-instar gerrids were placed in each of four compartments within 24 hours of hatching. Two of the compartments were maintained as control groups; the other two compartments were augmented with 20 wild gerrids let loose within the compartment. To prevent direct contact between the wild gerrids and those in the individual tubs, all tubs (control and high-density treatments) were fitted with a mesh lid.

All gerrids were checked and fed every day. Gerrids were assessed for gregarine infections within 24 hours of their imaginal moult. The total gregarine loads and gameocyst loads were transformed (log[x+1]) to correct for a skewed distribution and then analysed using a general linear model (proc GLM for unbalanced data). Planned linear comparisons were computed for the treatment mean against the control mean. The prevalence of gregarines in the treatments was analysed using Chi-square analysis (SAS, proc Freq).

During the course of these experiments the natural gregarine prevalence in the Meadow Pond *Gerrid* population was monitored for comparison (n=50; July 30, Aug. 9, Aug. 25). Samples of 50 adult gerrids were collected while travelling once around the edge of the pond.

3.3.7 Exposure to infective elements

To determine the source of gregarine infection in gerrids, host exposure to various possible infectious elements was manipulated. The hypotheses included direct uptake from the water, ingestion of aquatic prey containing oocysts and ingestion of aerial prey containing oocysts.

Eight treatments were designed to vary exposure to combinations of these elements. The treatments were applied in floating tubs (surface area of approximately 1.5 m^2 on George Lake). The gregarine prevalence was higher on George Lake (0.10) than on Meadow Pond (0.038) at the time; therefore the experiment was completed on George Lake. Treatments included all combinations of open or coarse-meshed top and closed, coarse-meshed or fine-meshed bottoms. In addition there were three closed-bottom tubs containing tap water and pond water, and one filled with tap water but exposed to surface tension contents through meshed slits at water level in the tub wall.

Gerrid eggs were collected from field-raised adults. The eggs were hatched on aerated tap water in the laboratory. First instar larvae were removed on the day they hatched and placed in one of the treatment tubs. Gerrids were fed laboratory-reared *S. bullata, ad libitum*. Gerrids were removed within 48 hours of their 5^{th} instar moult, and examined for gregarine infection.

3.3.8 Potential carrier prey

Samples (n=30) of bottom-feeding larval chironomids and larval mosquitoes were collected from Meadow Pond and examined for established gregarine infections. They are both abundant as gerrid prey items throughout the season and both have aquatic larval stages with the imaginal molt occurring at the water surface. Chironomid larvae were collected from pond sediments and mosquito larvae were collected from the pelagic zone with an aquatic net. These two groups were selected because they have the greatest potential to be a carrier of infective oocysts.

3.4 RESULTS

3.4.1 Retrieving gametocysts

No gametocysts were recovered from excreta from gerrids collected on Meadow Pond during high gregarine prevalence and kept in the laboratory over a two-week period.

3.4.2 Gametocyst development

Gametocysts were placed in three treatments to assess rate and extent of development in fresh water, a Ringer's control and a simulated gut. In all three treatments, gametocysts matured and released oocysts. However, the oocysts become active and released sporozoites only in Ringer's with gut extract (Table 3.1).

The rate of gregarine development varied between the three treatments. Oocysts were released within 24 hours in both of the Ringer's treatments and within 48 hours in the fresh water treatment (Figure 3.2). Sporozoites were released within 72 hours in the gut extract treatment.

Proportion of gametocysts that released oocysts also varied between treatments (Figure 3.2); only in the gut extract treatment did all ten gametocysts dehisce (Table 3.1). The gametocysts in the gut extract treatment also commenced oocyst release first and had the highest proportion of gametocysts maturing to release oocysts; this treatment was the only one to produce oocysts that became active and released sporozoites.

3.4.3 Water and Vertical transmission

No infections were incurred by larvae or adult gerrids raised on water with oocysts, nor on water taken from Meadow Pond. Likewise, no gerrids became infected in the laboratory habitat variation experiment. Also, one hundred offspring of infected females, but raised in non-infective conditions failed to become infected.

3.4.4 Transmission in field-varied habitat structures

In both of the two field experiments that tested how environmental structure affected gregarine infections, gregarine loads within and between treatments were characterised by an extremely high variability and by a wide range (Table 3.2).

In the first experiment, the food limited gerrids took significantly longer to develop and were significantly smaller than gerrids in the control treatment (Table 3.3a,b). The treatment x enclosure interaction term was significant for both total gregarine load (Table 3.4a) and gametocyst load (Table 3.4b), which complicates the interpretation of significant main effects. Neither the sex of the gerrid nor the enclosure in which it was raised affected gregarine loads. However, the main effect of treatment was significant in both analyses. The treatment linear contrasts were not significant for total gregarine load and thus significance of the main effect may be due to comparisons between experimental treatments, which are not of interest here (Table 3.4a). Food-limited gerrids, however, had a visibly higher prevalence than the control, with two out of the three replicates having a prevalence of 1.00 (Table 3.2, Figure 3.5). Gerrids of the food limited treatment also had significantly more gametocysts than the control group at the imaginal moult (Table 3.4b).

Load frequency distributions were not affected by treatments as there are no discernible patterns or trends between treatments, controls (Figure 3.3) or between treatments and frequency distributions of infections in simultaneous natural gerrid population of Meadow Pond (Figure 3.4).

Infected gerrids within the triplet treatment were randomly allocated throughout the individual tubs of three gerrids in enclosures 2 and 3 (Table 3.6). If gerrids transmitted gregarines to each other through direct contact, the number of tubs with 3 infected gerrids would be higher than expected; this is clearly not the case. In enclosure 4, there were more triplet tubs containing one infected gerrid and fewer triplet tubs containing two infected gerrids than expected. This suggests that the triplet treatment in enclosure 4 was associated with dispersed infections between tubs, for which there is no reasonable biological explanation.

In the second experiment, the treatment in which pond-reared gerrids were surrounded by infected gerrids (high density treatment), did not increase total gregarine load or gametocyst load (Table 3.5a,b), or gregarine prevalence (χ^2=0.60, d.f.=1, P=0.44, Figure 3.5).

A strong spatial effect was seen in gregarine prevalence at a scale of 6m. The control in experiment two (enclosure 8) had a gregarine prevalence approximately 60% lower than that of experiment one (enclosures 2,3,4) and the natural Meadow Pond population (Figure 3.5); however, within experiment one, in which enclosures were within 75 cm of one another, the main effect of enclosure had no effect on gregarine loads (Table 3.4a,b).

3.4.5 Carrier prey

No evidence of developing gregarines (trophozoites established on the epithelium) was found in dissections of either bottom dwelling chironomid larvae or mosquito larvae. Oocysts were not seen, but their presence in the guts of chironomid and mosquito larva cannot be ruled out conclusively, as they may have been overlooked in the gut matrix.

3.5 DISCUSSION

3.5.1 Gametocyst development

A. glabra sporulated within 24 hours under laboratory conditions at 20°C and near 100% humidity. Because the gametocysts were removed from the alimentary canal of the host rather than obtained immediately after they were formed, this time is a conservative estimate of the time for complete gametocyst development: from formation in the gut to oocyst dehiscence. This temperature is far below optima suggested by others. Moreover, the gametocysts sporulated relatively rapidly (24 hrs) in comparison to other reports, which vary from 24 to 110 hours for sporulation (Richardson and Janovy, 1990; Sengupta and Haldar, 1996 respectively).

Three lines of evidence strongly suggest that gametocysts of *A. glabra* develop and dehisce within the gerrid host and that oocysts are subsequently released into the environment. First, gametocysts in a simulated gut environment matured, and more rapidly than those placed in fresh water (Figure 3.2). Second, no gametocysts were excreted with frass by live gerrids placed in petridishes. Third, crescentic structures associated with dehisced gametocysts were regularly observed within the host alimentary canal (unpublished data, see Chapter 2). This is direct evidence that

gametocysts mature and dehisce within the host gut.

The release of oocysts within the host gut could greatly improve gregarine reproductive success. Gametocysts require high humidity and heat to develop, the combination of which may be rare upon exiting a semi-aquatic host as the pond environment is exposed and therefore dries rapidly. Within the host alimentary canal, the gametocysts would consistently develop at 100% humidity. Kozloff (1958) found that *Pyxinia crystalligera* could not develop at 100% humidity, however,*in vivo* gametocyst development does occur in other species (Beier and Craig, 1985; Chen and Ching-Hsiang, 1996). Several *Ascogregarina* spp. undergo gametogeny and sporogeny in the Malpighian tubules of their mosquito hosts; the gregarine is excreted as oocysts shortly after adult emergence (Beier and Harris 1983; Vávra, 1969).

The host gut may be the most environmentally stable and predictable place for gametocyst development in aquatic and semi-aquatic hosts. If gametocysts largely sporulate within the host gut, they have retained the ability to do so outside the gut, in fresh water as well.

3.5.2 Autoinfection

The question of whether or not autoinfection occurs is a separate consideration from whether or not *in vivo* sporulation occurs. Once it is clear that oocysts are released within the host gut, one must ask if they mature and release sporozoites in a hospitable region of the gut, thereby re-infecting or autoinfecting the host.

Autoinfection increases the number of individuals composing a parasite infection. A parasite that autoinfects its host avoids the life cycle bottleneck of extra-host stage survival as well as that of locating and infecting a new host. On the other hand, by increasing the size of the infection, autoinfection increases intra-specific competition within the host, as well as increasing the pathogenicity and therefore the probability of destroying the host (Frank, 1996). Autoinfection occurs in the Order Neogregarinida Grassé, 1953 in which gamonts undergo merogeny (Zizka, 1972). Consequently neogregarine infections generally have higher incidences and measurable pathogenicity (Tanada and Kaya, 1993).

Eugregarine infections are generally less severe and less pathogenic, in part because they are not thought to autoinfect their hosts. Eugregarines do not, by definition, undergo merogeny (Tanada and Kaya, 1993), so if present, autoinfection would occur

by completion of gamogeny and sporogeny within the epithelialated region of the host gut, resulting in production and attachment of new sporozoites.

With respect to the *Ascogregarina-Gerris* system specifically, maturation of gametocysts and release of oocysts within the gerrid gut would promote the symbiont. Autoinfection would allow the gregarine to maximise use of the current host environment. Also because gerrids can be cannibalistic, though not coprophagous, the release of viable oocysts within a host may also serve to spread the gregarine throughout the population. However, high infections seen in young instars of gerrids means that cannibalism is not a major route of transmission as well, most cannibalism occurs by adults on young instars (Klingenberg and Spence, 1996). Tieszen and Molyneux (1989) have demonstrated cannibalism as a possible means of transmission for trypanosomes in gerrids, however, the same limitations would apply to their system.

There is some evidence suggesting that autoinfection might occur with *A. glabra*, however, the balance of evidence is against it. First, oocysts in the simulated host environment released active sporozoites, thereby completing the reproductive cycle in an artificial host environment. This is largely inconclusive as it is possible that exposure to ambient light, a change in pH or ambient oxygen triggered dehiscence of gametocysts in all treatments, when this would not occur within the host naturally. Subsequently, having been signalled that it is outside the host and releasing oocysts, the gerrid gut extract would then trigger the release of sporozoites in the gut extract treatment only.

Second, gametocysts completed development in all treatments, but most rapidly in a simulated gut environment. Rapid development by gametocysts would increase the residency time of oocysts within the gut and therefore the likelihood that they were able to release sporozoites before passing from the epithelialated area of the gut. Gametocysts are extremely large in comparison to the host gut, and often get caught in front of constrictions in the gut preventing the host from eliminating them with frass. Once the oocysts have been released from the gametocysts, they are embedded in a highly viscous, gelatinous matrix which may reside in pockets and folds in the epithelial layer thereby further delaying elimination by the host. Therefore although oocysts of *A. glabra* require approximately 48 hours to mature and release sporozoites they may stay resident in the gut this long.

Third, large parasite loads and evidence of multiple infection events have been used

to suggest autoinfection in Odonates (Åbro, 1976). A strong bimodal distribution in gregarine size is often observed within gerrid adults (unpublished data); however, this distribution pattern also occurs in larvae that are younger than the predicted gregarine generation time, and therefore can not be accounted for by autoinfection. Furthermore, if autoinfection were a significant route of infection, one would see a strong bimodal effect in the gregarine load frequency distributions: a peak of gerrids with no infection and another peak with extremely high gregarine loads. Instead, the distributions are spread fairly smoothly from 0 to sporadic high loads suggesting a hyperbolic curve (Figure 3.4).

Finally, infected gerrids removed to a sterile laboratory environment would maintain gregarine infections by autoinfection, but this does not occur. This sum of evidence shows, at the least, that autoinfection is not a common or infection-sustaining occurrence in gerrids.

3.5.3 Other infection routes

Oocysts that are released into the environment must then find a suitable host and establish an infection (attaching to the epithelial cells of the alimentary canal). Two possibilities remain for the oocysts: they may be vertically transmitted or horizontally transmitted.

Vertical transmission

There was no evidence of *A. glabra* in the reproductive tract of *Gerris* spp.; infections were found exclusively in the alimentary canal. Gametocysts of *Ascogregarina chagasi* occasionally attach to and develop on the accessory glands of its host, releasing oocysts into the gland (Warburg and Ostrovska, 1991); offspring are then infected through ingestion of their egg case (Wu and Tesh, 1989). The fact that infections are not maintained between generations in lab cultures suggests that vertical transmission does not occur in *A. glabra*. In fact, vertical transmission did not occur in the lab study in which infected *A. glabra* females did not transmit infections to their offspring.

Direct and indirect horizontal transmission

Direct horizontal transmission, where parasite transmission is effected through direct contact between conspecifics, was tested by rearing individuals to adults in groups of three. This triplet treatment did not increase gregarine load. Neither did this treatment increase the chance of an uninfected gerrid housed with an infected bug, becoming infected (Table 3.6). Parasite transmission between conspecifics may occur in hosts with chewing mouthparts through grooming or touching (group feeding, defecating, diapause aggregation); however, it is unlikely in gerrids because of their piercing and sucking mouthparts, and has not been documented in other gregarine-host systems either. Many oocysts have complex external architecture that is clearly designed to adhere to animal bodies or vegetation (Åbro, 1976), however *A. glabra*, and *Ascogregarina* spp oocysts in general appear smooth, except for polar plugs (Levine, 1988).

Indirect horizontal transmission through accidental ingestion of oocysts free in the environment is the most cited route of infection, likely because it is the simplest and easiest to verify. In aquatic hosts, this is through ingestion of oocysts directly from the water or of oocysts attached to food (Beier and Craig, 1985). Gerrids do ingest water from the water column when feeding (Pollard and Spence, unpublished observations), and trypanosomes infecting gerrids are thought to be taken up directly from the water column (Tieszen and Molyneux, 1989). However, the gregarine oocysts sink in fresh-water (unpublished data) and so they are unlikely to be picked up in this way. Waterstriders failed to become infected when held on water containing viable oocysts, pond water, or in high-density treatments. This suggests that the infection mechanism for *A. glabra* requires some other component to operate.

Gregarines must necessarily be excreted from the host with frass at some stage, whether as gametocysts or oocysts. Because gerrids commonly rest on surface vegetation, it is very likely that gregarines are deposited there. Also, surface vegetation may serve to retain the gregarines near the water surface rather than sink into the pond. Porter (1909) suggests that larvae drink adult frass from leaf surfaces, thereby ingesting trypanosomes . However, in laboratory tests, gerrids reared with *Potomogeton* sp. leaves failed to become infected; in the field experiments, gerrids raised with *Potomogeton* sp. leaves did not exhibit higher (or lower) gregarine infection loads (Table 3.4a,b) or prevalence (Figure 3.5). Therefore the presence of surface aquatic

vegetation does not seem to facilitate infection of *Gerris* spp. with gregarines.

One possibility not experimentally considered in this study is that gregarines are released as sporozoites in frass droplets which are then relatively quickly consumed by younger instars. The vegetation used in these experiments was taken from Meadow pond and sporozoites on the surface would have dried up and lost the ability to infect. This mechanism has been suggested for trypanosome transmission (Porter, 1909) but has never before been described in a gregarine system.

Prey vector

A simple and efficient way for predatory gregarine hosts to become infected by gregarines is for them to ingest oocysts with their prey. Because gerrids have piercing and sucking mouthparts, any prey-vector must necessarily ingest the oocysts in order to transmit them to the gerrid host. In this manner, the prey could act as a concentration mechanism for the gregarine as they eat oocysts and accumulate them in their guts. This study suggests that neither vertical transmission nor direct ingestion of oocysts from water, vegetation, or conspecifics occurs; therefore the only remaining possibility is that gerrids ingest oocysts while feeding on prey.

Oocysts deposited or released on the water surface will sink to the bottom of vessels in the laboratory (personal observation). Oocysts of *Ascogregarina* species may get temporarily caught on vegetation as they sink, however, they have no external architecture (spines) to keep them in place, therefore, these oocysts likely come to rest near the bottom of the pond. As gerrids reside almost all the time at the surface of the pond, it would require another organism that is a common gerrid prey item to bring the infective oocyst back up to the surface where it could infect gerrids.

Using the life history of the gerrid host, one can define several aspects of the life history of the carrier species. The carrier prey species would have to deliver infective oocysts within its body (presumably the alimentary canal) to the water surface for the gerrid to ingest. Therefore, the carrier must ingest oocysts at the bottom of the pond. These carriers might migrate to the surface for oxygen, to avoid predators or for their imaginal moult. Either beneath the surface film, or above it, invertebrates would be susceptible to predation by gerrids (Anderson, 1982).

From data about the prevalence and seasonal patterns of the gregarine infection in gerrids (unpublished data), one can define more characteristics of the carrier host. The

gregarine population is at a low prevalence within the gerrid population immediately upon gerrid emergence from diapause. The prevalence then gradually climbs over the season as new infections are introduced into the gerrid population throughout the summer. Gregarine prevalences in gerrids on Meadow Pond have approached 100% over two out of four years (unpublished data). Any carrier prey population would, therefore, have to be feeding (ingesting oocysts) and emerging over the length of the summer, likely by having multiple generations over the season; otherwise, the carrier host population could consist of a suite of species that fit these criteria.

An unexpected and telling observation was the enormous variation in prevalence between gerrids raised at the same time but in different locations of the pond (Figures 3.1 and 3.5). Since the gregarine prevalence in the natural gerrid population was near 1.00 over the course of these experiments, this observation must reflect a spatial phenomenon within the pond, detected by the use of the enclosures. If the carrier species is unevenly distributed on the pond bottom, and rises approximately straight up for its imaginal moult, the gregarine load in captive gerrids would reflect this local effect. This enormous spatial variation in gregarine prevalence seems to rule out transmission by water and proves that some second level of structure is required for this gregarine's transmission to its host. Further, this observation corroborates the theory of infection by a prey item, one that is unevenly distributed on the bottom of the pond and suggests a high specificity of carrier prey rather than a large suite of species serving in this capacity. Gerrids are generalised predator-scavengers and eat both water-trapped aerial prey and aquatic prey (Spence and Andersen, 1994). *A. glabra* is clearly not transmitted to gerrids through terrestrial prey. Laboratory trials using light trap caught prey produced no infections in gerrids. Also, it is unlikely that aerial prey are the oocyst carriers because insects tend to evacuate all gut content derived from the aquatic system at the imaginal moult, and therefore would not be carrying infective gregarines. Aquatic prey were also investigated as a possible oocyst carrier. This was not tested directly in the laboratory, and field experiments failed to resolve this due to an extremely low background prevalence level at George Lake.

Unfortunately, no studies have been published on the diet composition of *G. buenoi*. Both notonectids and mosquitoes are abundant at Meadow Pond and have been cited as important food sources in other *Gerris* species (Selvanayagam, 1986). Although notonectids are an important food source for some gerrids they can not be

the primary source of infective gregarines as they do not bottom feed at any stage. Gerrids also feed on mosquitoes, particularly larval and pupal mosquitoes as teneral mosquitoes are harder to detect and catch (Nummelin, 1988; Selvanayagam, 1986). Mosquito larvae would be a good oocyst carrier as species feed throughout the water column and migrate near the surface before emerging as adults. Chironomids are an equally good candidate as a carrier prey, and are also extremely abundant in Meadow Pond. However, inspections of larvae of both groups yielded no evidence of established gregarine infections, nor oocysts, although oocysts are unlikely to be detected amongst the lumen matrix.

3.5.4 Susceptibility of food limited hosts

Food-limited gerrids showed a higher gregarine prevalence and larger numbers of gametocysts than gerrids under control conditions. This group also had a significantly longer development time and a smaller body size (Table 3.3a,b) which indicates that these treatment gerrids had suboptimal assimilation of food. This could be explained by hunger triggering increasing the rate of infection for example through a behavioural change. More likely though, this observation is more directly caused by some change in the alimentary canal. Insects naturally have a very rapid turnover rate in the epithelial layer in the alimentary canal, however the regular sloughing of cells also reduces the number of protozoan parasites attached to gut cells (Kurup, 1966). An increase in gametocyst prevalence in food limited gerrids is likely a direct result of a reduction in the epithelial turnover rate, however the cause of this is not immediately clear. Food limited gerrids may be unable to mount as effective an immune response as well, such as by prematurely releasing epithelial cells that have been infected (Tanada and Kaya, 1993). This would allow gregarines to establish infections more easily, and allow a larger proportion of them to complete their life. However, the observed effect could also be the result of a natural reduction in the rate of epithelial cell shedding due to food stress, which would incidentally result in longer gregarine tenancy and therefore a higher prevalence and a larger number of gregarines reaching the gametocyst stage.

3.5.5 Conclusions

There is good evidence that gametocysts of *A. glabra* develop within the gerrid host. Autoinfection thus remains a possibility, as viable oocysts are released within the host; however, there is insufficient evidence to prove it occurs and it can not be the sole explanation for the levels of prevalence observed. Testing this hypothesis would require careful laboratory work, and probably require the ability to infect hosts in the lab.

There is good evidence that gregarines are transmitted to gerrids through a prey item that carries oocysts up from the bottom sediments. The vector, however, remains unknown.

There is clearly a strong spatial effect in gregarine infection within Meadow Pond and this may well provide a hand hold from which to further investigate the transmission of the *G. buenoi* - *A. glabra* system.

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Table 3.1. The rate and extent of gametocyst development under three artificial treatments.

Treatment	Proportion dehisced	Oocysts active	Sporozoites released
Fresh water	0.7	-	-
Ringer's solution	0.8	-	-
Ringer's with gut extract	1.0	+	+

+ present, - absent

Table 3.2 Summary statistics for gregarine loads and prevalences of field transmission treatments.

Treatment	Proportion infected: Weighted mean (replicates)	Mean Gregarine Load (n)	Standard Error	Range: Gregarine Load
Control	0.86 (1.00, 0.82, 0.78)	60.1 (51)	10.7	0-297
Food limited	0.98 (1.00, 1.00, 0.94)	79.4 (49)	11.8	0-342
Triplet	0.74 (0.92, 0.67, 0.64)	57.8 (175)	7.0	0-494
Vegetation	0.86 (0.90, 0.88, 0.78)	49.7 (55)	8.2	0-290
Control	0.26 (0.27, 0.25)	63.8 (23)	26.6	0-568
Wild	0.17 (0.20, 0.14)	42.0 (29)	10.9	0-259

Table 3.3a. Effect of treatment, sex, enclosure and an interaction term on the development time of *G. buenoi* in experiment 1, 1994

Source	d.f.	Mean square	F	P	
Treatment	3	409.97	46.16	<0.01	
Sex	2	92.35	10.4	<0.01	
Enclosure	1	29.13	3.28	0.04	
Treat.*Enclosure	6	23.43	2.64	0.02	
Error	310	8.88			
Contrasts:					LS Mean
Food limited	1	615.14	69.26	<0.01	33.17
Triplet	1	21.44	2.41	0.12	27.31
Vegetation	1	4.15	0.47	0.49	27.66
Control					28.06

Table 3.3b. Effect of treatment, sex, enclosure and an interaction term on the adult size of *G. buenoi* in experiment 1, 1994

Source	d.f.	Mean square	F	P	
Treatment	3	2.98	40.72	<0.01	
Sex	2	56.1	766.23	<0.01	
Enclosure	1	0.03	0.44	0.64	
Treat.*Enclosure	6	0.13	1.8	0.10	
Error	306	0.07			
Contrasts:					LS Mean
Food limited	1	5.46	74.59	<0.01	7.41
Triplet	1	<0.01	0.05	0.83	7.90
Vegetation	1	0.23	3.1	0.08	7.79
Control					7.89

Table 3.4a. Results of GLM analysis on log-transformed total gregarine load of field transmission treatments (food limited, triplet, vegetation) with treatment contrasts.

Source	d.f.	Mean square	F	P
Sex	1	0.119	0.20	0.66
Treatment	3	2.966	4.96	<0.01
Enclosure	2	0.180	0.30	0.74
Treatment*Enclosure	6	2.878	4.81	<0.01
Error	317	0.598		
Contrasts				
Food limited	1	2.033	3.40	0.07
Triplet	1	1.327	2.22	0.14
Vegetation	1	<0.001	<0.01	0.99

*Gregarine load was calculated by summing all trophozoites, gamonts and twice the number of gametocysts. This total was transformed as log (x+1).

Table 3.4b. Results of GLM analysis on log-transformed gametocyst load of field transmission treatments (starvation, triplet, vegetation) with treatment contrasts.

Source	d.f.	Mean square	F	P
Sex	1	0.066	0.15	0.70
Treatment	3	2.064	4.62	<0.01
Enclosure	2	0.162	0.36	0.70
Treatment*Enclosure	6	2.217	4.96	<0.01
Error	317	0.447		
Contrasts				
Food limited	1	2.478	5.54	0.02
Triplet	1	0.178	0.40	0.53
Vegetation	1	0.264	0.59	0.44

*Gametocyst load was calculated by doubling the number of gametocysts in the host and then transformed as log (x+1).

Table 3.5a. Results of GLM analysis on log transformed total gregarine load* of high-density treatment.

Source	d.f.	Mean square	F	P
Sex	1	0.501	0.76	0.39
Treatment	1	0.193	0.29	0.59
Compartment (treatment)	2	0.358	0.55	0.58
Error	47	0.657		

*Gregarine load was calculated by summing all trophozoites, gamonts and twice the number of gametocysts. This total was transformed as log (x+1).

Table 3.5b. Results of GLM analysis on log transformed gametocyst load* of high-density treatment.

Source	d.f.	Mean square	F	P
Sex	1	0.501	0.76	0.39
Treatment	1	0.193	0.29	0.59
Compartment (treatment)	2	0.358	0.55	0.58
Error	47	0.657		

*Gametocyst load was calculated by doubling the number of gametocysts in the host and then transformed as log (x+1).

Table 3.6 Results of the χ^2 analysis testing for even distribution of infected *G. buenoi* in the high-density treatment.

Enclosure No.	Frequency of tubs with 0,1,2, or 3 gerrids infected (expected value†) 0	1	2	3	Prevalence in sample (n)	d.f.	χ^2	P
Enclosure 2	1 (0.9)	4 (4.6)	8 (7.5)	4 (4.0)	0.67 (51)	3	0.115	0.999
Enclosure 3	0 (0.0)	0 (0.4)	5 (4.1)	15 (15.6)	0.92 (60	3	0.604	0.896
Enclosure 4	0 (0.4)	8 (3.2)*	5 (8.3)*	6 (7.1)	0.64 (57)	3	8.929	0.030

† Expected number of tubs having n/3 infected gerrids=(probability of n/3 gerrids being infected in a tub)(number of tubs in treatment) e.g. for enclosure 2, 3/3 gerrids infected: $(0.62)^3(17)=4.1$; e.g. for enclosure 4, 2/3 gerrids infected: $(0.72)^2(0.28)(3)(19)=8.3$

* produced a high $\chi 2$ value

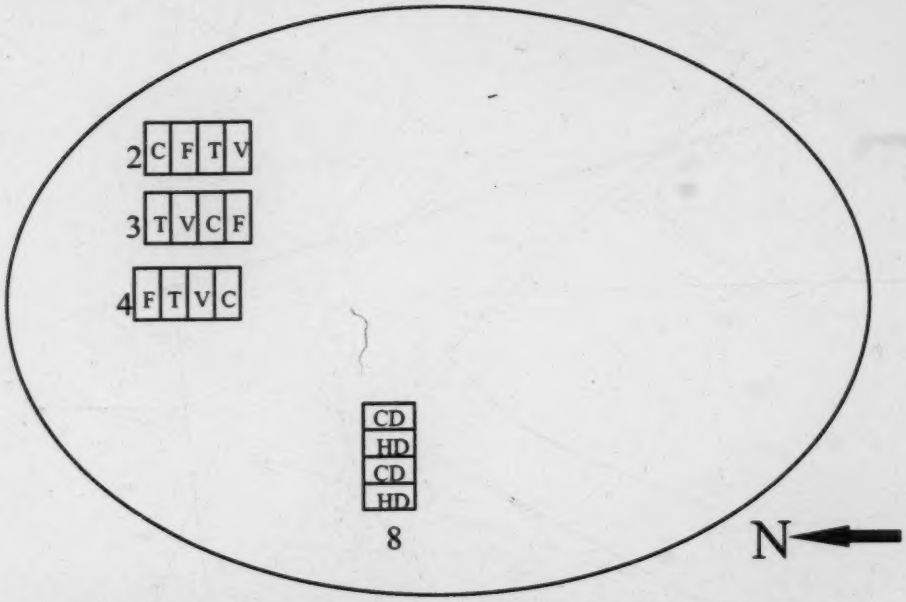

Figure 3.1. Diagram of enclosure layout. Enclosures 2,3,4 housed treatments C=control, F=food limited, T=triplet, V=vegetation. Enclosure 8 housed treatments CD=control, HD=high density.

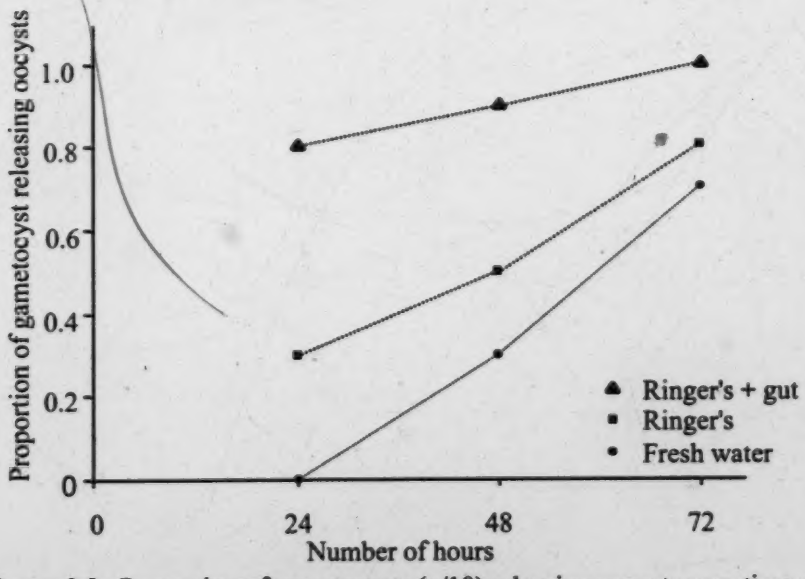

Figure 3.2. Proportion of gametocysts (x/10) releasing oocysts over time, under three different treatments (ringer's and gut, ringer's, fresh water).

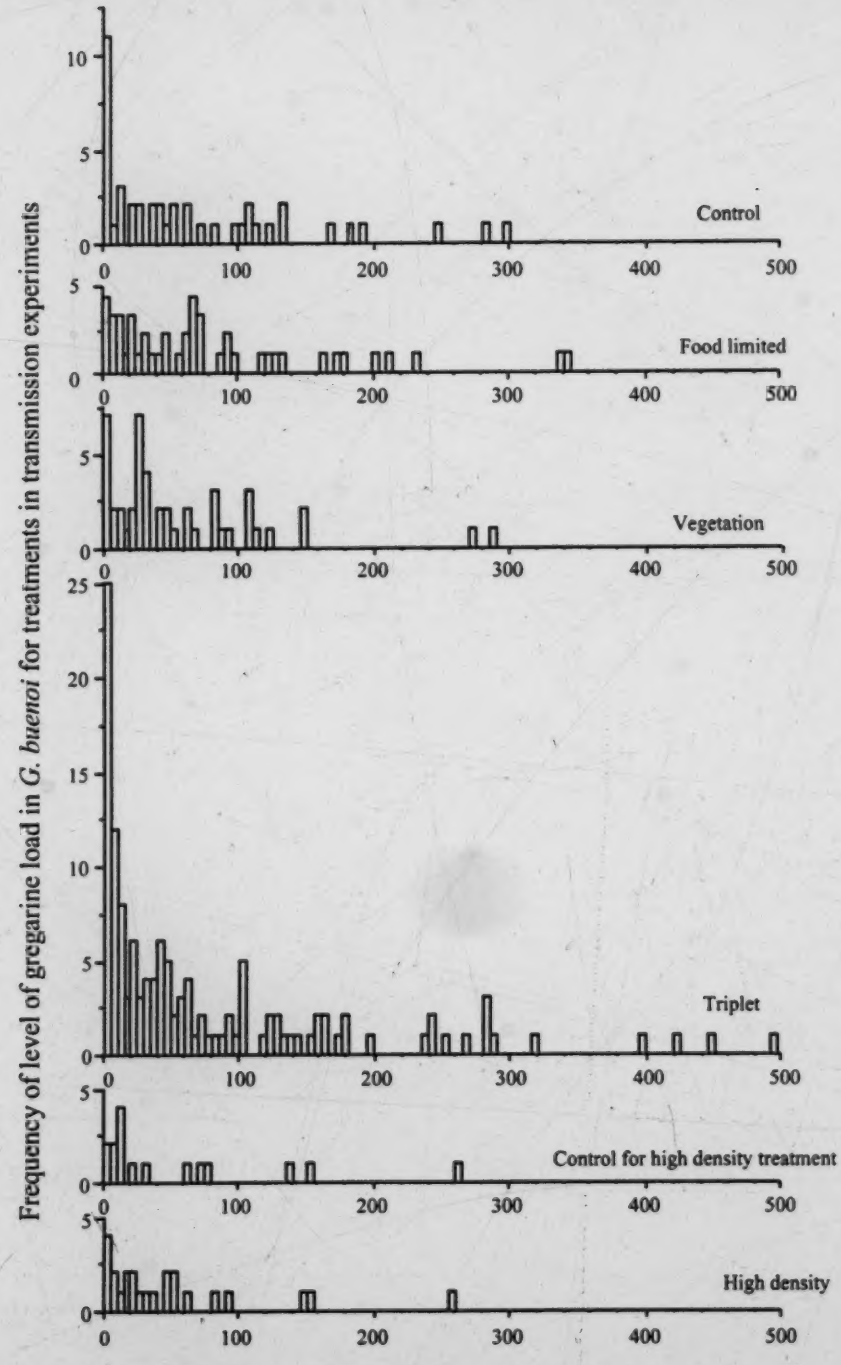

Figure 3.3. Frequency distributions of gregarine load in *G. buenoi* split by treatment.

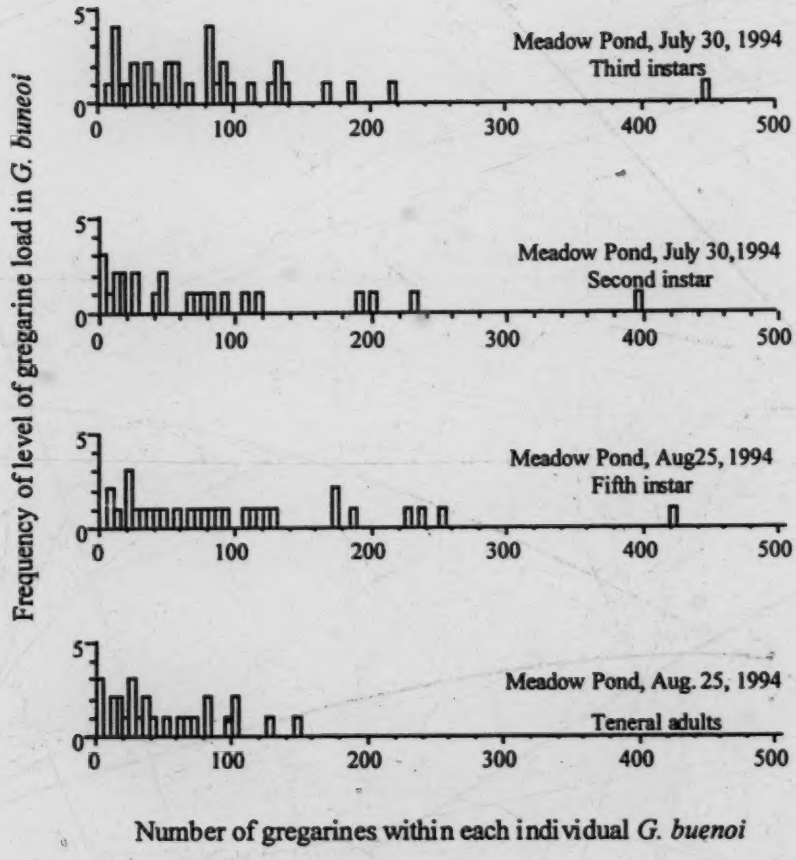

Figure 3.4. Gregarine load frequency distributions from natural *G. buenoi* populations on Meadow Pond

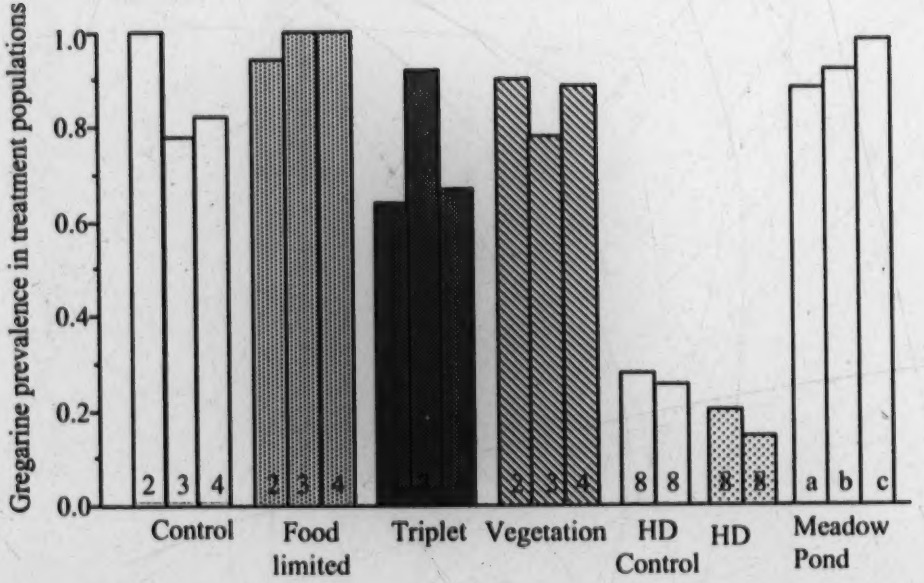

Figure 3.5. Gregarine prevalence in field experiments 1 and 2, and on Meadow Pond for the duration of the experiment. The numbers 2-4 (experiment 1) and 8 (experiment 2) on the treatment bars denote the enclosure number; HD=High density. The Meadow Pond sample "a" was collected on July 30, "b" on August 9, "c" on August 25.

Chapter 4

Pathogenicity of *Ascogregarina glabra*: measuring the effects on *Gerris buenoi* Kirkaldy (Hemiptera: Gerridae) life history.

4.1 SYNOPSIS

The pathogenicity of *Ascogregarina glabra* for *Gerris buenoi* was investigated under both field and laboratory conditions in central Alberta. *A. glabra* had no clear effect on adult size or larval development time of *G. buenoi*. Further, there was no significant effect of the gregarine on gerrid weekly fecundity on either direct breeding summer generation females (1997) or post-diapause females (1998) in either laboratory or field trials. In 1998, however, natural gregarine prevalences were uncharacteristically low and thus reduced the power of the analyses.

There was a weak trend for diapause-bound males with high gregarine loads to take flight less frequently than those with lower loads. This observation did not hold for female gerrids.

No pathogenic or beneficial impacts of *A. glabra* on *G. buenoi* were detected at the life history level. Thus despite the large and impressive infections of this gregarine observed in Alberta, there is no evidence that *A. glabra* is an important selective agent acting on its primary host *G. buenoi*.

4.2 INTRODUCTION

The pathogenicity of gregarines has generally been investigated at the host life history level and largely with the hopes of using this group in biological control programs, particularly as applied to vectors of human diseases (Beier and Craig, 1985). Gregarines, and particularly the eugregarines, however, are not highly pathogenic in their natural hosts (Beier and Craig, 1985; Tanada and Kaya, 1993).

Pathogenicity by eugregarines is thought to be derived primarily from physical damage to the epithelial cell layer (Åbro, 1971; Harry, 1970; Lipa, 1967). Generally, gregarines cause their most severe pathology when they develop intracellularly, or cause a breach in the intestinal wall, which can result in secondary bacterial infections (Tanada and Kaya, 1993). Several authors have also noted that due simply to the size of the infections, gregarines must inhibit absorption and movement of food through the alimentary canal (Åbro, 1971; Klingenberg et al., 1997; Lipa et al., 1996).

Low level pathogenicity is harder to detect, but is of great interest in terms of the life history, population and evolutionary biology of the host species. Documented effects of eugregarines on their natural hosts ranges from mild pathogenicity with respect to host growth (Harry, 1967,1970; McCray et al., 1970; Siegel et al., 1992), longevity (Dunkel and Bousch, 1968; McCray et al. 1970; Siegel et al., 1992), and vigour (Simmons, 1990; Simmons and Zuk, 1992; Zuk, 1987) to positive effects on host growth, where the parasite is thought to provide some nutrient to the host (Sumner, 1933 and 1936 but see critique by Harry 1967).

Infections of *A. glabra* in gerrids are very conspicuous (>600 individuals in one host) and can be extremely prevalent (up to 1.00) (Barrington Leigh, unpublished). *A. glabra* develops entirely extracellularly (see Chapter 2) and attaches to epithelial cells via a smooth rather than hooked mucron (epimerite). The attachment mechanism is likely similar to that described by Lucarotti (in press). This gregarine does rupture its host cell upon release and retains its mucron (Chapter 3).

The life history components of the hosts are plastic and enable gerrids to adapt to features such as continuity of habitat (Kaitala, 1988; Spence, 1989) and food availability (Kaitala, 1987) with different reproductive (Blanckenhorn, 1991; Rowe and Scudder, 1990), dispersal (Spence, 1981), and diapause (Andersen, 1982) strategies. By influencing the amount of overall resources available to an individual, or a population, a pathogenic parasite may affect life history traits (such as growth, fecundity,

and overall vigour) and therefore play a significant role in population dynamics. In a previous study, this gregarine has shown no measurable effect on growth parameters of field-raised gerrids fed *ad libitum* and I have been unable to demonstrate a heritable component of susceptibility or resistance to infection among hosts (Klingenberg et al., 1997).

In order to assess potential impacts of *A. glabra* on *G. buenoi*, the growth, fecundity and propensity for flight were investigated under varying gregarine loads. The results are discussed in the broader context of host population biology.

4.3 METHODS

4.3.1 Site description

All *Gerris buenoi* in this study were obtained from Meadow Pond at the University of Alberta George Lake Field site (114° 06' W, 53° 57' N, 90 km NW of Edmonton, Alberta). All field experiments were run on Meadow Pond. Meadow pond is a permanent man-made pond that supports a diverse fauna, including a large population of *G. buenoi* as well as several other gerrid species.

4.3.2 Assessment of gregarine infection

All gerrids were assessed for gregarine infections by dissection. The gerrids were decapitated and dissected while submerged in Ringer's solution. The remaining alimentary canal was removed and placed on a glass slide in Ringer's solution. The gut was examined under a dissecting microscope and then opened along its length. The preparation was then covered with a glass cover and examined under a compound microscope to assess the number and stage of all gregarines.

Two measures of infection size were used for analyses. Total gregarine load was calculated by totalling the number of trophozoites and gamonts and then adding twice the number of gametocysts, as each gametocyst is formed by two mature gamonts. Secondly, the number of gametocysts was used and, although a conservative measure it is a better estimator of the number of feeding gregarines present throughout the development of the larvae because each gametocyst present represents two gamonts that fully developed over 16-20 days in the host (Chapter 2). Both total gregarine

load and gametocyst load data were log transformed (log[x+1]) to correct for a skewed distribution.

During dissections of *G. buenoi*, observations of the general condition of the host and its alimentary canal were made.

4.3.3 Gregarine impact on *G. buenoi* growth parameters

The effect of gregarine infection on two important growth parameters, adult size and larval development time, were investigated by analysing *G. buenoi* collected from Meadow Pond and gerrids that were raised in enclosures on Meadow Pond. All analyses were conducted separately for males and females given some evidence that *G. buenoi* males and females respond differentially under stress (Spence, 1986).

Field reared gerrids

An experiment was designed to investigate how the gregarine is transmitted (Chapter 3). Individual first instar *G. buenoi* (n=360) were raised in small tubs kept in three large enclosures, under four treatments: a control, food limitation, a group provided with vegetation and a triplet treatment where three gerrids were raised together (Chapter 3). The larvae were checked and fed everyday except in the food limitation treatment, in which they were fed every other day. Within 24 hours of the imaginal moult, individuals were removed to the laboratory. Total body length was measured using a pair of vernier calipers and development time calculated in days. Gerrids were assessed for gregarine infections in the laboratory.

Gerrid size and development time were linearly regressed on both measures of gregarine load (SAS, proc REG). For the analyses using total gregarine load, the four treatments were pooled, as treatment did not explain total gregarine load in an overall analysis (Chapter 3). Individuals in the food-limited treatment were removed for the analyses of gametocyst load as this treatment showed a significantly higher gametocyst load as well as reduced growth compared with the other treatments (Chapter 3).

The host growth parameters were also logistically regressed (using maximum likelihood estimation; SAS, proc CATMOD) on gregarine prevalence and gametocyst prevalence. For this and all subsequent analyses, a gerrid was considered infected

during its larval development if it harboured any stage of gregarine, or the crescentic structures derived from the gametocyst (see Chapter 3) which indicate that gametocysts were recently present. Individuals from the food-limited treatment were excluded in the logistic regression of gametocyst presence on growth parameters.

Total gregarine load was linearly regressed on both growth parameters for the food-limited treatment alone to test for the possibility that food stress causes gerrids to be more susceptible to pathogenic effects of gregarines.

Collected adult gerrids

Adult gerrids (n=50) were collected from Meadow Pond every two weeks between July 17 and August 25 in 1994. These collections represent the summer generation, as few overwintered gerrids survive past July 15 in the field (John Spence, personal communication). Gerrids were stored at 5°C until measured, dissected and assessed for gregarine infection.

The data were analysed both split and pooled by date because a preliminary analysis (SAS, proc GLM; Table 4.1) showed that total gregarine load was significantly different across collection dates.

Adult size was linearly regressed on both total gregarine load and gametocyst load. Adult size was also logistically regressed on gregarine presence and gametocyst presence.

4.3.4 Gregarine impact on female reproductive efficacy

The effect of gregarine infection on reproductive efficacy was investigated under both field and laboratory conditions during 1997 and 1998. I measured reproductive effort of gerrids using weekly fecundity and proportion of those eggs that hatched (sometimes referred to as fertility rate). Weekly fecundity was used because it responds most dramatically to reduced food levels (Rowe and Scudder, 1988) and because gregarine infections, being transitory, can not be estimated over the reproductive life span of the female. An assessment of gregarine infection load at time t is considered a good estimate of the infection the host has carried over the previous week (t-1week).

Direct breeding females

Thirty fifth instar females were collected from Meadow Pond on June 20, 1997 and fed *ad libitum* until their imaginal moult. Adult females were paired with males and held in individual tubs on Meadow Pond (missing males were replaced within 24 hours). Females were checked and fed every other day. Most natural prey was excluded because, as above, the tubs were kept in large, screened enclosures on the pond. Each female was provided with a strip of polystyrene on which to lay eggs. One week from the commencement of egg laying, females were assessed for gregarine infection. Number of eggs laid and proportion hatched was assessed 3 weeks after collection.

Measures of female fecundity and the proportion of eggs hatched were linearly regressed on measures of gregarine load and female size. These two measures of reproductive success were also logistically regressed on gregarine presence and gametocyst presence.

Post-diapause breeders

Female gerrids (n=60) were collected from Meadow Pond on 27 April, 1998, paired with males and set up in individual tubs on Meadow Pond in the same manner as above. A parallel group of females (n=120) was established up in the laboratory and treated identically.

To test the hypothesis that gregarine load impacts females differently over their reproductive life span, groups of females were dissected after laying for 1, 2, 3, or 4 weeks. Both the average number of eggs laid per week, and the number of eggs laid in the week immediately prior to dissection were used in the analyses.

Fecundity measures were logistically regressed on gregarine presence. Analyses of gregarine load and gametocyst presence could not be conducted because of low prevalence. Analyses were done separately for field and laboratory components.

4.3.5 Gregarine impact on flight propensity

The hypothesis that gregarines affect gerrids' propensity to take flight either directly, by adding to the host mass, or indirectly, acting physiologically, was tested.

In 1997 fifth-instar gerrids (n=60) collected from Meadow Pond were placed in

field enclosures and fed *ad libitum*. Within 24 hours of the imaginal moult, adults were removed to another compartment during the teneral period to allow for full maturation of the flight muscles (Andersen, 1973). On the eleventh day after imaginal moult, individuals were taken to the laboratory and held at 5°C before being tested for flight. Gerrids were taken in groups of twenty from 5°C to an enclosure where they were placed in individual tubs in direct sunlight. The air temperature ranged from 34-36°C. Every two minutes, all gerrids that had flown out of their containers onto the ground or walls of the enclosure were collected into a container marked for that two-minute interval. This was repeated for 10 two-minute periods (20 minute total). All gerrids that did not fly during this 20-minute period were considered non-flyers. Body length and sex were recorded for each individual before it was assessed for gregarine infection. The flight muscles of each gerrid were assessed for maturity, primarily by their size and by comparison with immature flight muscles. Time of take-off was linearly regressed on both gerrid size and total gregarine load. Presence/absence of flight was logistically regressed on total gregarine load

4.4 RESULTS

4.4.1 General observations of gregarine infections in gerrids

There was no obvious external indication of gregarine infection in *G. buenoi* and the alimentary canal of infected gerrids exhibited no differences in tissue colouration. However, when disturbed in dissections, trophozoites detached from their host cells and had a "hood" of epithelial cell tissue attached to the epimerite, constituting proof that cells are ruptured by trophozoite release (Chapter 2). Some dissected gerrids had large numbers (>100) of loose epithelial cells in their alimentary canal although this feature was not limited to hosts infected by gregarines.

In severe gregarine infections, the first and third gut ventricles were noticeably distended and misshaped due to the presence of large numbers of gamonts and gametocysts. In many hosts gamonts appeared to "pile up" at the juncture between the first and second ventricle and also between the third ventricle and the hind gut. This gregarine forms conspicuous infections in gerrids at least partly because the gamont stage is very large, on average more than one tenth of the length of the host.

4.4.2 Gregarine impact on growth

All measurements of gregarine infection clearly showed that there was no effect on growth of reared gerrids. In field reared gerrids, total gregarine load (trophozoites, gamonts plus twice the number of gametocysts) did not predict larval development time (Figure 4.1a) or adult size in females (Figure 4.1b). Some males with very high gregarine loads had lengthy development times, however, the overall effect was not statistically significant (Figure 4.1a). High total gregarine loads significantly depressed male body size, however, the effect was small and explained little of the variation (Figure 4.1b). Gametocyst load did not predict either development time or adult size of gerrids reared in exclosures in Meadow Pond (Figures 4.2a,b). Some males and females with heavy infections appeared to take longer to develop, however this was counterbalanced by a few mildly, or uninfected gerrids of both sexes which also developed slowly (Figure 4.2a). Gregarine presence and absence was also not a significant predictor of either development time or adult size (Table 4.2). Even food limited gerrids did not exhibit an effect of either total gregarine load or gametocyst load on either development time or adult size (Table 4.3).

For gerrids collected from Meadow Pond, correlation between gregarine infection loads at dissection and the level of the infection during their development period may be weak as the time since the imaginal moult would have an unknown and possibly large range. However, the advantage of sampling from a natural population is that the gerrids have been exposed to competition for resources and the other rigours of life in the field.

In pooled analyses total gregarine load ranged from 0 to 631, yet no significant predictive relationship was evident between measures of gregarine load and adult size; in fact, half of the regression slopes were negative and the remainder positive (Table 4.4). However, there was one exception. For males from July 17, a high gregarine load significantly predicted an increase in size (a slope of 0.24) and explained 29% of the variation in male size (Table 4.4, Figure 4.3). For females from August 25 in the unpooled analyses it appeared that there was a significant positive relationship, however, this result depends solely on two points for infected gerrids (Figure 4.4). The pooled analyses of body size and gametocyst suggested a positive relationship, however the relationship was weak (Table 4.4).

In general, gregarine prevalence started off low in the spring and reached almost

1.00 by the end of the season (Table 4.5). As a result, logistic regressions of gregarine presence for collections from July 17 to August 25 had very low power due to the rarity of uninfected gerrids in the samples. A similar caution is necessary for the analyses of gametocyst presence, as several collection dates have very low prevalences, in fact, some could not be conducted (Tables 4.4, 4.5). In the pooled analysis of gametocyst prevalence among females, there was a marginally significant, negative effect on size (Table 4.6), however, examination of the data shows that the analysis is likely driven by one outlier and low numbers of infected gerrids (Figure 4.5a). In the pooled males, size was clearly not affected by gametocyst load (Table 4.6, Figure 4.5b). Therefore, none of the logistic regressions provided a significant relationship between gregarine presence and adult size (Tables 4.4, 4.5).

4.4.3 Gregarine impact on reproduction

Neither fecundity nor hatch rate was affected by total gregarine load or by gametocyst load in direct breeders during 1997 (Table 4.7a,b, Figure 4.6a, b). Logistic regressions of fecundity and hatchability confirmed that gregarine presence had no effect on fecundity (Table 4.8). These findings were further confirmed by both the field and laboratory experiments with post-diapause breeders during 1998 (Table 4.9). Although these findings are consistent with those of the previous season (1997), they must be interpreted with extreme caution as the number of gerrids infected with gregarines are extremely low.

4.4.4 Gregarine impact on flight propensity

Total gregarine load did not significantly decrease the propensity of gerrids to take flight (Figure 4.7). However, when flight was treated as a binary variable, there was a suggestion that higher gregarine loads inhibited flight in male gerrids (this is not the case in females; see Figure 4.8a). This trend was more pronounced when the outlier was removed from the logistic regression analysis (Table 4.10, Figure 4.8b).

All individuals in this study exhibited large, mature indirect flight muscles therefore no gerrids were excluded from this analysis. Therefore even extremely high gregarine loads did not inhibit the metabolically expensive growth of these muscles.

4.5 DISCUSSION

4.5.1 General observations of gregarine in the host:

Much qualitative evidence suggests that gregarines must be pathogenic to gerrids either at the tissue (destruction of cells by attachment and detachment), or organ (stretching of alimentary tissues, obstructing food passage and absorption) level. It is reasonable to infer that these would weaken the host's physiological system directly and increase the host's mass thereby reducing overall host vigour. However, I have been unable to convincingly demonstrate any such effects.

Several authors have cited damage to the host alimentary canal, in particular to the epithelial layer (Åbro, 1971; Harry, 1970; Lipa, 1967), and attributed it to gregarine infection. These claims must be interpreted cautiously as a very good understanding of the natural state of the alimentary canal is logically important for comparison but is rarely provided. In *G. buenoi*, the possible evidence of physical damage to the epithelial layer can not be distinguished from descriptions of the natural state (Kurup, 1966). Large numbers of epithelial cells found in the third ventricle could be due to a host immune response (Tanada and Kaya, 1993), or result from a surge of holocrine excretion, which "is accomplished by a wholesale separation and discharge of a section of the epithelium" (Kurup, 1966). This process can also result in the appearance of a depleted epithelial layer on one side of a gut cross-section, which is sometimes cited as evidence of gregarine damage (e.g. Figure 2b in Åbro, 1971). The alimentary canal has a very high regenerative capacity with epithelial cell life expectancies being as short as 4 days in some insects (Tanada and Kaya, 1993); this may alleviate or negate some of the impact of the gregarine infections. Therefore, the gut condition in *G. buenoi* can not be considered abnormal, or attributed to gregarine infection without further investigation.

Nonetheless, infections of *A. glabra* in gerrids exhibit an imposing presence in the alimentary canal. Mature gamonts are on average more than one-tenth the length of the host (Chapter 2); intense infections of over 600 gregarines in one host have been reported; the first ventricle is sometimes obviously distended by the gregarine load (Chapter 2); the entrance to the second ventricle can be completely blocked by an infection and epithelium obscured from the lumen content by gregarines. Therefore, I must concur with authors of papers about other host-eugregarine systems (Åbro,

1971, Harry, 1970) - it is difficult indeed to conceive that such gregarines have no pathogenic effect on their hosts.

In this investigation, I have assumed that significant pathogenicity of *A. glabra* on *G. buenoi* should produce effects analogous to those of food limiting treatments because the main source of gregarine pathogenicity would be a decrease in the rate and amount of nutrient assimilation in the gut.

4.5.2 Life history parameters of *G. buenoi*

Growth of *G. buenoi*

Two measurements of growth were examined in *G. buenoi*. Body size is used as a central measure in life history theory and it is generally accepted that a larger size results in better survival, mating advantages and higher reproductive output (Roff, 1992). In *G. buenoi*, size is generally uncorrelated with measures of fecundity (Klingenberg and Spence, 1997), but adult body size decreases when raised in food-limited regimes (Chapter 3; Klingenberg et al., 1997; Spence, 1986), and therefore body size should be a reasonable measure of pathogenicity of a gut parasite. Shorter development time is thought be an advantage for larval gerrids as they are vulnerable to both predation and cannibalism (Spence, 1986, 1989). Furthermore, development time should also be sensitive to a significant reduction in nutrient availability as it increases significantly in *G. buenoi* raised in food-limited regimes (Chapter 3; Klingenberg et al. 1997; Spence, 1986).

In general, the analyses strongly support the notion that gregarines have no impact on host growth, however, there are some notable exceptions. Negative impacts on growth may be statistically obscured by a small number of uninfected gerrids (males in Figure 4.1a; males and females in Figure 4.2a). Furthermore, gregarine load does predict a smaller adult size of males in one sample (Figure 4.1b). All but one of the analyses of the reared gerrids, however, show that there is no significant effect of gregarine infection on growth.

From these results, one could imagine that gerrids are only affected under specific conditions. Several investigators having found no clear impact of gregarines on otherwise healthy hosts have examined the impact of gregarines on compromised hosts.

For example, some hosts show more sensitivity to gregarine infection under food limited conditions (Dunkel and Bousch, 1969; Zuk, 1987). However, food limitation was not associated with a significant regression of gregarine load (total gregarine load, gametocyst load) on either development time or adult size. This is strong evidence that *A. glabra* does not have a significant impact on adult size or development time under the normal range of conditions experienced by this *G. buenoi* population.

There was one case in which high total gregarine load was strongly associated with significantly increased adult size (Table 4.4, Figure 4.3). Because these gerrids were collected rather than reared under controlled conditions, one can not assume this result to mean that gregarines benefited the gerrids nutritionally. This result could be a reflection of the amount of food procured which may be a strong predictor of the number of infective oocysts the gerrid is exposed to; therefore, successful foragers may have higher gregarine loads by virtue of their success.

Fecundity of *G. buenoi*

In order for a parasite to noticeably affect the amount of energy a female is able to allocate to production of eggs, the energy lost to the parasite must be greater than that provided by the plasticity of other energy sources (e.g., wing muscle histolysis in *G. thoracicus*, Kaitala, 1988). *Gerris* spp. exhibit an interesting trade-off between daily fecundity and longevity so that females in sub-ideal habitats (low food treatments) may spread their reproductive effort out over both time and space without reducing life time fecundity (Kaitala, 1987; Rowe and Scudder, 1988; Spence, 1989). Therefore, when examining the daily fecundity of infected and uninfected females, we are really asking if the parasite deprives the host of sufficient resources to push the fecundity-longevity trade-off towards lower daily fecundity and greater longevity.

Females in this study were fed every other day, constituting a limited food regime (Chapter 3). Even though under food stress, the regressions of fecundity on gregarine loads in both 1997 and 1998 clearly show that there is no impact of gregarine load on weekly fecundity, although results for 1998 must be interpreted with caution due to low gregarine prevalences.

Flight of *G. buenoi*

Arguably the most metabolically expensive activity (per unit time) undertaken by macropterous gerrids, is flight; therefore flight may be the most sensitive measure of pathogenicity of this apparently benign gregarine. However, there was no effect of gregarine load on propensity to fly in *G. buenoi* (Table 4.10). After removal of an outlier, the logistic regression of fliers versus non-fliers on gregarine load suggests that males with high gregarine loads are less likely to take flight (Figure 4.8b). Of course, the outlier was a notable exception to this trend, taking flight fairly rapidly (6 minutes) and carrying the largest gregarine load of all (>300) and this reduces confidence in the generalisation.

Perhaps a more relevant measurement would be flight endurance as both the ability to fly and distance capability influence the effectiveness of dispersal and therefore mating and diapause patterns (Kaitala, 1990; Spence, 1989). We can examine the possibility that gregarines deter flight or reduce migration capability in gerrids through reasoning. Firstly, reduced dispersal in infected individuals would tend to concentrate gregarine populations in specific sites, characterised by factors allowing efficient gregarine transmission (see Chapter 3) and one which provides a fairly stable, permanent habitat for *G. buenoi*. In smaller and more temporary habitats gregarine populations would become locally extinct, as infected gerrids would be less capable of migrating out of the site and less likely to recolonize these habitats. This pattern is seen in the *G. buenoi* study population on Meadow Pond as it consistently shows a disproportionally high gregarine prevalence compared to surrounding habitats (Barrington Leigh, unpublished).

Secondly, highly infected gerrids would be selected against in poor or unpredictable habitats as their ability to migrate to better habitats when necessary would be compromised; highly infected gerrids may not be measurably disadvantaged in high quality and stable habitats as other life history traits appear unaffected.

Thirdly, if high gregarine loads affect flight, other measures of vigour such as competition for territory (and therefore food and mates) should be compromised in proportion to the energy cost of the activity. The samples of gerrids collected from Meadow Pond have competed for resources and therefore represent a range of successful competitors in each collection. The size of these gerrids, reflective of the level of food procured during development, does not show any consistent relationship

with gregarine load (Tables 4.3).

Overall, these arguments suggest that there is no marked effect of gregarine load on propensity to fly, however, the possibility that gregarines reduce migratory ability is intriguing and should not be ruled out until the flight endurance of infected gerrids has been tested.

4.5.3 Host-parasite theory

Conventional host-parasite theory contends that parasites relying on their hosts for transmission should evolve to be benign, and that only poorly adapted parasites are highly virulent or pathogenic to their hosts (Anderson and May, 1982; Bull, 1994, Ewald, 1983; Klassen, 1992). However Frank (1996) more recently argued that virulence is a trade-off with the efficiency of transmission, meaning that the more the parasite relies on the host for transmission, the less virulence it will evolve.

The *A. glabra* - *G. buenoi* system seems largely consistent with the classical school of thought. These gregarines are not highly dependent on their host for transmission as it is not vertically, but horizontally transmitted; furthermore, oocysts are a highly environmentally resistant extra-host stage that do not rely on the host once released. Therefore, the gregarine only needs 16-20 days residence within its host in order to propagate (Chapter 3). Competition for resources within the host also elicits the evolution of more virulent parasites. There appears to be a fair amount of competition for resources within the host given the large gregarine infections although we have observed no statistical interaction between gregarines and other internal symbionts (Klingenberg et al, 1997). According to current models of host-parasite relationships, *A. glabra* should be an extremely virulent parasite. Yet by all accounts presented here, *A. glabra* is benign to its primary host *G. buenoi*, except for the weak trend suggesting a negative impact on flight, thus precluding significant coevolution between host and parasite, and suggesting this gregarine is not pathogenic enough to exert a selective pressure on the host.

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Table 4.1. Effect of sex and collection date on the total gregarine load in *G. buenoi* collected from Meadow Pond. Eight collections of 50 gerrids were made between May 20 and August 25, 1994.

Source	d.f.	Mean square	F	P
Collection date	7	14.267	30.88	<0.01
Sex	1	0.062	0.13	0.72
Date*sex	7	0.775	1.68	0.11

Table 4.2. Logistic regressions of *G. buenoi* growth and size on gregarine presence for field experiment, 1994.

	Sex	Sample size	d.f.	$\chi 2$	P
Larval development time					
Total gregarine load	f	164	1	0.51	0.48
	m	159	1	0.07	0.78
Gametocyst load	f	143	1	1.69	0.19
	m	136	1	0.18	0.67
Adult body size					
Total gregarine load	f	163	1	0.04	0.83
	m	156	1	0.11	0.73
Gametocyst load	f	141	1	<0.01	0.96
	m	133	1	0.12	0.73

Note: the food limitation treatment was excluded for analyses of gametocyst load

Table 4.3. Results of linear regressions of *G. buenoi* growth and size on gregarine loads for food limited treatment analysed by sex.

	Sex	Sample size	d.f.	F	P
Larval development time					
Total gregarine load	f	18	1	0.03	0.86
	m	21	1	0.01	0.92
Gametocyst load	f	18	1	0.12	0.74
	m	21	1	0.06	0.81
Adult body size					
Total gregarine load	f	18	1	<0.01	0.98
	m	21	1	<0.01	0.99
Gametocyst load	f	18	1	1.26	0.28
	m	21	1	2.87	0.11

Table 4.4. Linear regression of *G. buenoi* size on measures of gregarine load for collections from Meadow Pond, 1994.

	Sex (n)	Slope (S.E.)	R^2	F	P
Total gregarine load					
July 17	f (35)	0.11 (0.09)	0.04	1.50	0.23
	m (16)	0.24 (0.10)	0.29	5.67	0.03*
July 30	f (22)	-0.10 (0.07)	0.08	1.74	0.20
	m (25)	0.01 (0.08)	<0.01	0.01	0.91
August 9	f (23)	-0.14 (0.10)	0.09	2.05	0.17
	m (26)	-0.08 (0.09)	0.04	0.94	0.34
August 25	f (27)	-0.03 (0.08)	0.08	0.10	0.76
	m (21)	-0.02 (0.14)	<0.01	0.02	0.88
All dates pooled	f (107)	<0.01 (0.04)	<0.01	0.01	0.93
	m (88)	<0.01 (0.05)	<0.01	<0.01	0.97
Gametocyst load					
July 17	f (35)	0.11 (0.16)	0.01	0.48	0.49
	m (16)	0.26 (0.14)	0.19	3.29	0.09
July 30	f (22)	-0.11 (0.16)	0.02	0.45	0.51
	m (25)	-0.09 (0.13)	0.02	0.45	0.51
August 9	m (26)	0.09 (0.17)	0.01	0.24	0.63
August 25	f (27)	0.40 (0.17)	0.19	5.86	0.02*
	m (21)	0.02 (0.26)	<0.01	<0.01	0.95
All dates pooled	f (107)	0.15 (0.09)	0.03	2.86	0.09
	m (88)	0.15 (0.08)	0.04	3.28	0.07

* significant effect; no gametocysts present in females from August 9

Table 4.5. Logistic regressions of *G. buenoi* size on gregarine prevalence for collections from Meadow Pond, 1994.

Collection date	Sex	Prevalence	Slope (S.E.)	$\chi 2$	P
July 17	f	0.85	-1.01 (1.13)	0.81	0.37
	m	0.94	-10.58 (8.34)	1.61	0.20
July 30	f	0.95	-1.83 (4.33)	0.18	0.67
	m	0.92	1.39 (2.88)	0.23	0.63
August 9	f	0.91	3.30 (2.81)	1.38	0.24
	m	0.92	3.80 (2.80)	1.84	0.18
August 25	f	0.96	-1.61 (4.32)	0.14	0.71
	m	1.00			
All dates pooled	f	0.92	-0.33 (1.08)	0.09	0.76
	m	0.94	1.31 (1.45)	0.82	0.36

Table 4.6. Logistic regressions of *G. buenoi* size on gametocyst prevalence for collections from Meadow Pond, 1994.

Collection date	Sex	Prevalence	Slope (S.E.)	$\chi 2$	P
July 17	f	0.29	-1.05 (1.07)	0.96	0.33
	m	0.50	-2.52 (2.16)	1.36	0.24
July 30	f	0.23	0.87 (2.20)	0.15	0.69
	m	0.20	1.86 (2.48)	0.56	0.45
August 9	f	0.00			
	m	0.15	-1.39 (2.04)	0.46	0.50
August 25	f	0.07			
	m	0.14	-0.28 (1.80)	0.02	0.88
All dates pooled	f	0.16	-1.87 (0.94)	4.02	0.04*
	m	0.23	-1.21 (0.84)	2.08	0.15

* significant effect

Table 4.7a. Linear regressions of fecundity and hatchability on total gregarine load and body size from field trial in 1997.

Variables	Sample size	Slope (S.E.)	d.f.	t	P	Full model			
						R^2	Mean square	F	P
Fecundity in week 1									
Total gregarine load	23	-4.91 (20.79)	1	-0.24	0.82	0.0378	15.30	0.04	0.97
Body size	23	-1.62 (25.05)	1	-0.06	0.95				
Hatchability									
Total gregarine load	23	-0.01(0.08)	1	-0.14	0.89	0.0141	<0.01	0.06	0.94
Body size	23	-0.03 (0.10)	1	0.47	0.79				

Table 4.7b. Linear regressions of fecundity and hatchability on gametocyst load and body size from field trial in 1997.

Variables	Sample size	Slope (S.E.)	d.f.	t	P	Full model			
						R^2	Mean square	F	P
Fecundity in week 1									
Gametocyst load	23	-15.23 (11.01)	1	-1.38	0.20	0.18	313.6	0.98	0.41
Body size	23	1.55 (19.62)	1	0.08	0.94				
Hatchability									
Gametocyst load	23	0.03 (0.05)	1	0.61	0.56	0.12	<0.01	0.62	0.56
Body size	23	-0.10 (0.09)	1	-1.04	0.33				

Table 4.8. Logistic regressions of fecundity measures on gregarine presence for direct breeders, 1997.

	Sample size	Prevalence	Slope (S.E.)	d.f.	$\chi 2$	P
Total gregarine presence:						
First week	23	0.91	-0.07 (0.08)	1	0.78	0.38
Proportion hatching	23	0.91	9.7 (17.10)	1	0.33	0.57
Gametocyst presence:						
First week	23	0.52	<-0.01 (0.03)	1	0.01	0.92
Proportion hatching	23	0.52	-6.68 (6.21)	1	1.16	0.28

Table 4.9. Logistic regressions of fecundity measures on gregarine presence for post diapause breeders, 1998.

	Sample size	Prevalence	Slope (S.E.)	d.f.	$\chi 2$	P
Laboratory						
Average weekly fecundity	79	0.09	-0.03 (0.04)	1	0.65	0.42
Fecundity in final week	79	0.09	<0.01 (0.03)	1	0.07	0.80
Field						
Average weekly fecundity	56	0.36	0.07 (0.07)	1	1.07	0.30
Fecundity in final week	56	0.36	0.02 (0.04)	1	0.29	0.59

Table 4.10. Results of logistic regression of flight on total gregarine load

Source	Sex	Slope (S.E.)	d.f.	$\chi 2$	P
Gregarine load	f	0.34 (0.79)	1	0.19	0.66
	m	1.23 (1.05)	1	1.36	0.24
Gregarine load*	m	5.07 (3.06)	1	2.74	0.0980*

* outstanding outlier removed

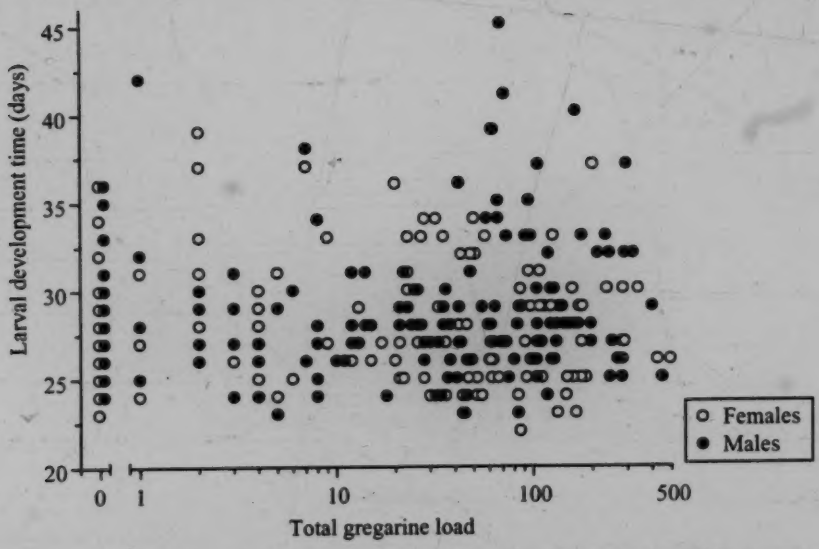

Figure 4.1a. Relationship between total gregarine load and larval development time of *G. buenoi* raised on Meadow Pond, 1994. Total gametocyst load is calculated by totalling all trophozoites, gamonts, and twice the number of gametocysts. For females, the regression equations is Y=-0.23X+28.00, P=0.48, R^2=<0.001. For males Y=0.39X+28.29, P=0.31, R^2=0.007. Where X = log (total gregarine load +1).

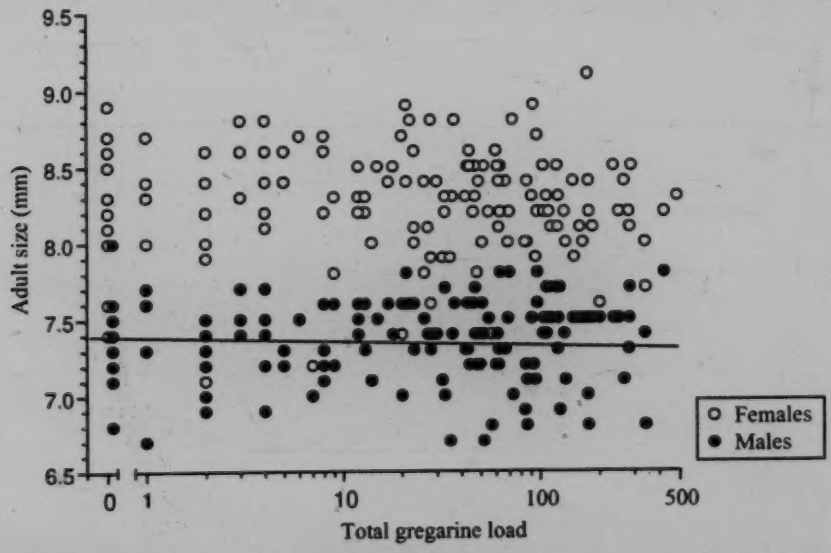

Figure 4.1b. Relationship between total gregarine load and adult size of *G. buenoi* raised on Meadow Pond, 1994. Total gametocyst load is calculated by totalling all trophozoites, gamonts, and twice the number of gametocysts. For females, the regression equations is Y= 0.01X+8.25, P=0.78, R^2=0.0030. For males Y= -0.06X+7.45, P=0.03, R^2=0.0300. Where X = log (total gregarine load +1).

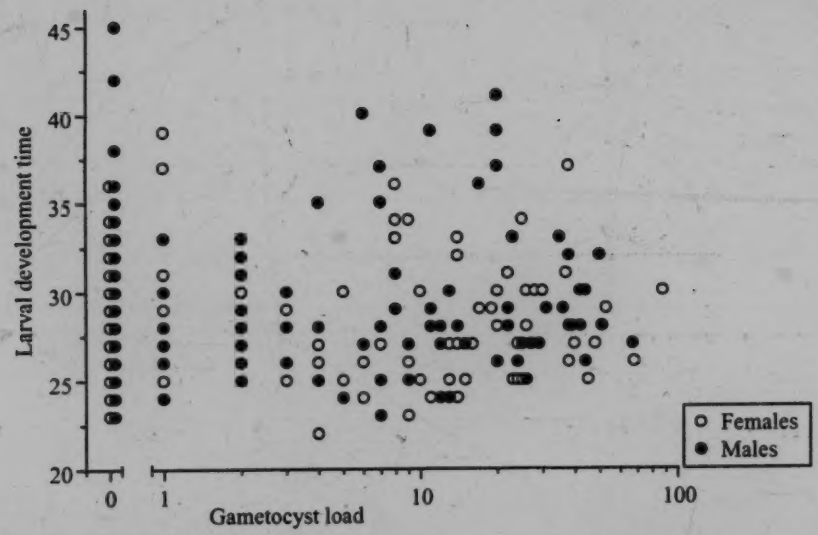

Figure 4.2a. Relationship between gametocyst load and larval development time of *G. buenoi* raised on Meadow Pond, 1994. The individuals in the food limited treatment were excluded from this linear analysis. For females, the regression equation is Y=0.20X+27.02, P=0.63, R^2=0.0017. For males Y=-0.05X+27.94, P=0.91, R^2=0.0001. Where X = log (gametocyst +1).

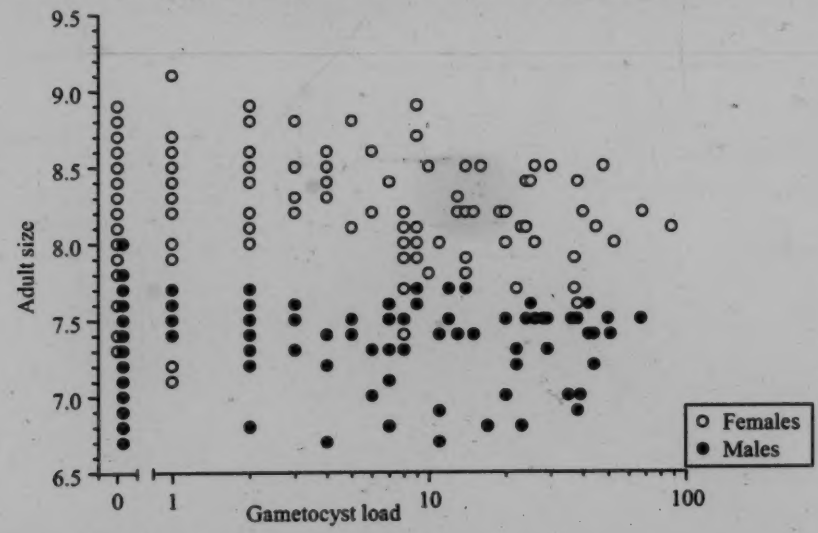

Figure 4.2b. Relationship between gametocyst load and adult size of *G. buenoi* raised on Meadow Pond, 1994. The individuals in the food limited treatment were excluded from this linear analysis. For females, the regression equations is Y=-0.05X+8.33, P=0.30, R^2=0.0079. For males Y=-0.02X+7.45, P=0.55, R^2=0.0027. Where X = log (gametocyst +1).

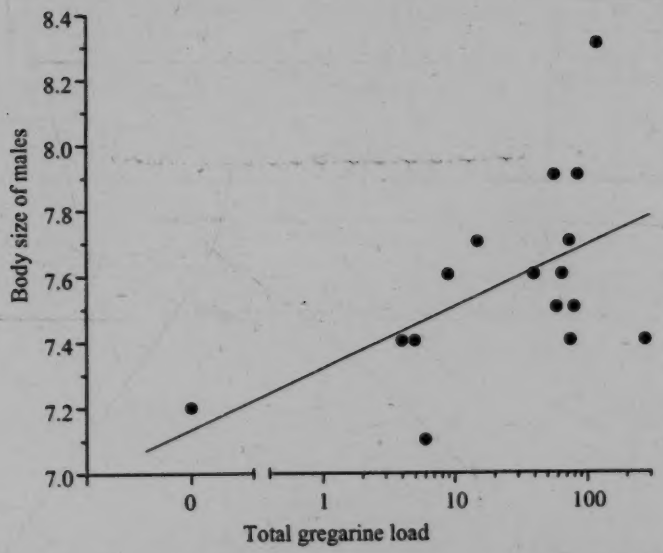

Figure 4.3. Results of a linear regression of gregarine load on adult body size of male gerrids collected from Meadow Pond July 17, 1994. Y= 0.24 (log gregarine load +1) +7.22, F=5.67, d.f.=1, P=0.0321,

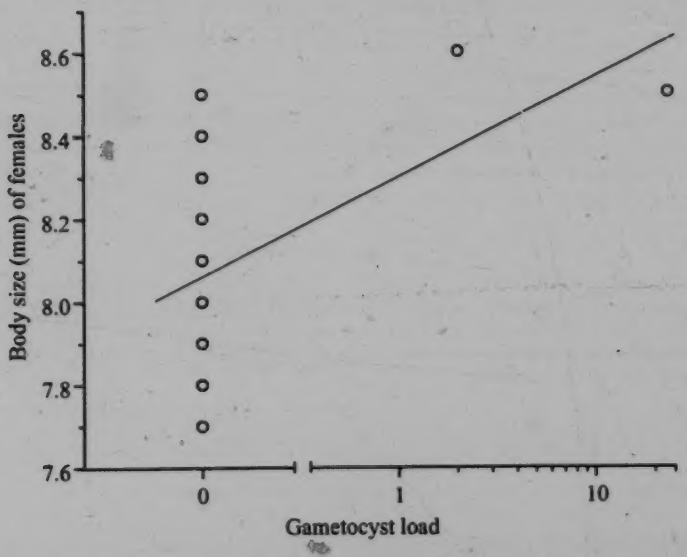

Figure 4. 4. Results of a linear regression of gametocyst load on adult size of females collected from Meadow Pond on August 25, 1994. Y= 0.40 (log gametocyst load +1) + 08.06, F=5.86, d.f.=1, P=0.0230

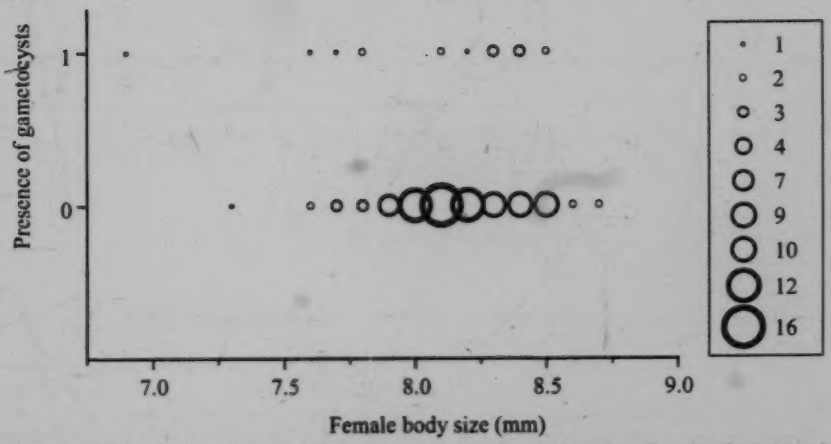

Figure 4.5a. Distribution of gametocyst presence in summer generation females from Meadow Pond collections in 1994. Gametocyst presence significantly reduces female body size (Table 4.5), however, examination of this figure shows that this is likely an artefact. The legend shows the number value for each size of circle.

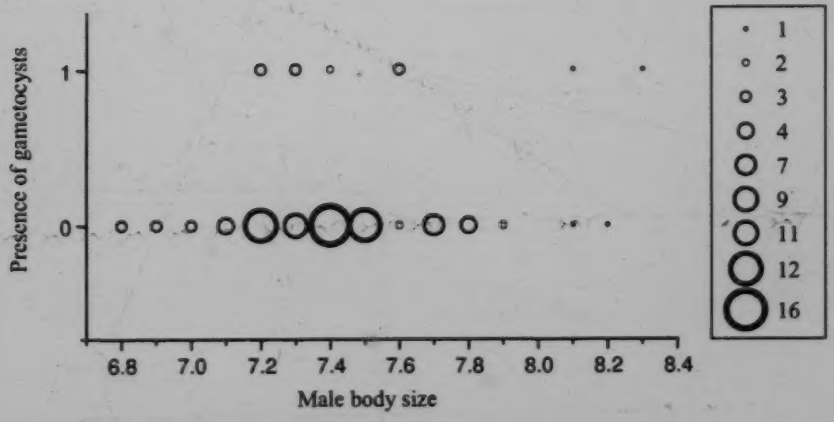

Figure 4.5b. Distribution of gametocyst presence in summer generation males from Meadow Pond collections in 1994. Gametocyst presence does not affect male body size (Table 4.5). The legend shows the number value for each size of circle.

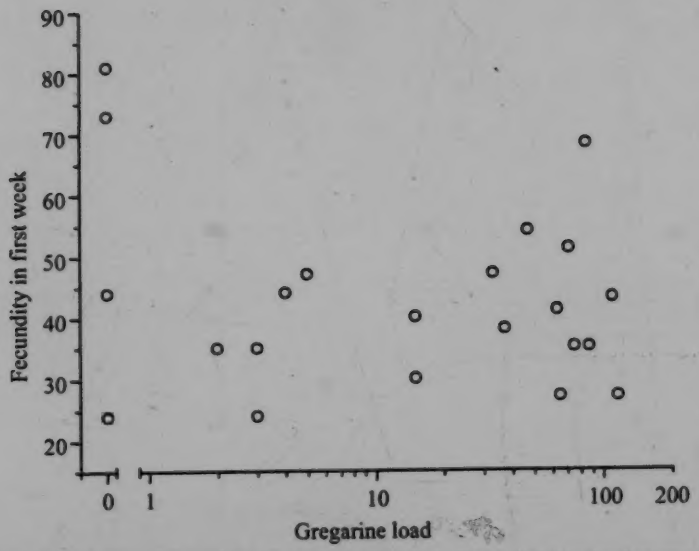

Figure 4.6a. The relation of gregarine load on the number of eggs laid in the first week by *G. buenoi* females housed on Meadow Pond, 1997.
The linear regression on total gregarine load is Y= -4.90X - 1.62(size) + 66.23, P=0.97, R^2=0.0082.
The linear regression on gametocyst load is Y= -15.23X + 1.55 (size) + 44.65, P=0.41, R^2=0.1786.

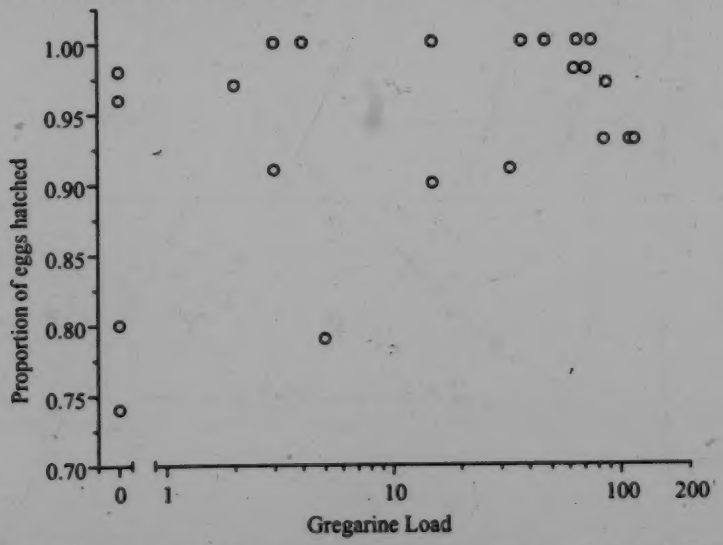

Figure 4.6b. Relation of gregarine load and proportion of eggs hatched in *G. buenoi* females housed on Meadow Pond, 1997.
The linear regression on total gregarine load is Y= -0.01X - 0.03(size) + 1.18, P=0.94, R^2=0.0040.
The linear regression on gametocyst load is Y= 0.03X - 0.10(size) + 1.72, P=0.55, R^2=0.1219.

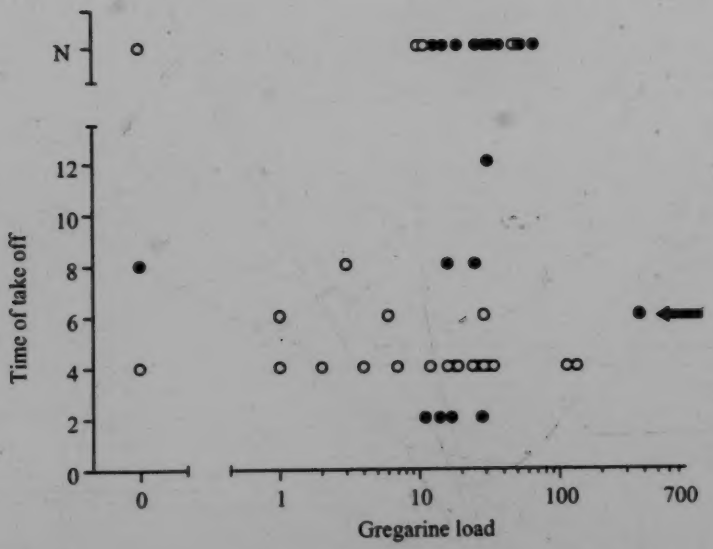

Figure 4.7. The relationship between gregarine load and the time gerrids took flight. Gerrids that did not fly within 20 minutes are denoted by "N" at the top of the Y-axis. One male, considered an outlier [372, 6] is denoted by a solid arrow and was removed for re-analysis.
Females (open circles): Y= -0.22X + 0.20 (size) + 3.06, P=0.84, R^2=0.0187
Males (filled circles): Y= -2.11X - 1.13 (size) + 17.33, P=0.63, R^2=0.1220;
outlier excluded: Y= -3.47X - 0.70 (size) + 15.24, P=0.53, R2=0.1909.

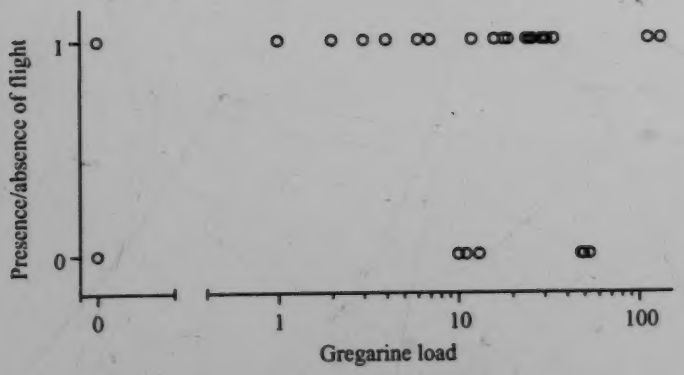

Figure 4.8a. Distribution of gregarine load in flying and non-flying females

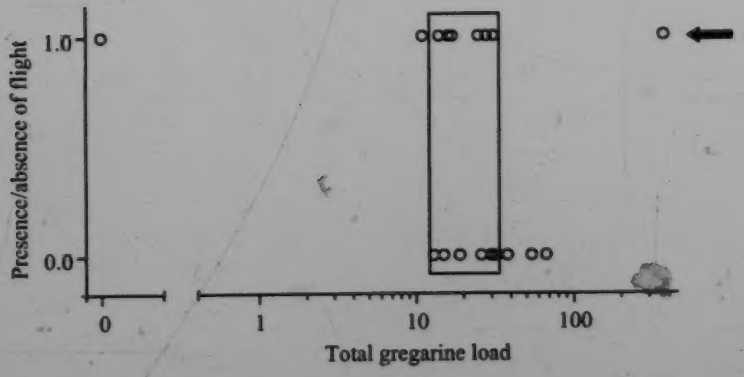

Figure 4.8b. Distribution of gregarine load in flying and non-flying males. One male, considered an outlier [372, 6] is denoted by a solid arrow and was removed for re-analysis. The box denotes the overlapping zone of gregarine load between fliers and non-fliers with the outlier removed.

Chapter 5

CONCLUSIONS

5.1 Summary

In this thesis, I have described a new eugregarine, *Ascogregarina glabra* (Apicomplexa: Eugregarinorida) from the alimentary canal of a *G. buenoi* population in central Alberta, Canada. I have placed *A. glabra* in the Family Lecudinidae, and the Genus *Ascogregarina* (previously part of Genus *Lankesteria*) as it has a simple mucron, spatulate gamonts, spherical gametocysts, and it occurs in insects (distinguishing feature between Genera *Lankesteria* and *Ascogregarina*) and because it does not remotely fit other generic descriptions (see Levine, 1988; Ostrovska et al., 1990).

It is early, considering our knowledge of *A. glabra*, to contemplate its evolutionary origin; however, one must note that 9 of 16 ascogregarine species occur in mosquito species (Levine, 1988), which are extremely common in aquatic systems as larvae, and compose a component of gerrid prey (Nummelin, 1988). This host-predator relationship provides an obvious passage for an adventitious symbiont. Molecular investigations into gregarine phylogeny could presumably elucidate these relationships (McManus and Bowles, 1996).

Understanding the mode of transmission is crucial for a general understanding of host-parasite systems. Furthermore, the means of transmission is considered an important factor in modeling the virulence of a parasite (Anderson and May, 1982; Frank, 1996). Most ascogregarine hosts become infected by ingesting oocysts from the water column oocysts, which are deposited by egg-laying females (Chen et al., 1997; Garcia et al., 1994; Sulaiman, 1992; Walker et al., 1987; Walsh and Olson, 1976). Few are vertically transmitted (Wu and Tesh, 1989) and only one case of transmission via a prey species has been documented among gregarines (Åbro, 1971).

I suggest that *A. glabra* is transmitted through aquatic prey, however, this is not proven; rather, most other possibilities have been eliminated: vertical transmission,

ingestion from the water, from conspecifics, or from vegetation. Furthermore, although *A. glabra* released infective oocysts within the host, these did not autoinfect the host, but rather passed out into the environment.

Several reports of eugregarine infections in insects have claimed significant loads on the hosts that appear to block food passage, obscure epithelial absorptive surface area and, in some cases, damage the epithelial layer (Chapter 4, Åbro, 1976; Harry, 1967). Detecting this apparent pathogenicity at the organismal level, however, has proven very difficult. When no impact is detected on vigorous hosts, both food-limited target hosts, and non-target hosts are studied (Dunkel and Bousch, 1969; Walsh and Olsen, 1976; Zuk, 1987).

Infections of *A. glabra* were benign in *G. buenoi* as measured by growth, fecundity and the ability of an individual to take flight. Gregarine loads did not positively or negatively impact larval development time, adult size or female fecundity under *ad libitum* or food limited treatments.

The data do suggest that larger gregarine loads inhibit the initiation of flight, however this effect is subtle. The flight endurance of gerrids was not investigated in this study, which would be most relevant in assessing the effect of gregarines on their ability to migrate between sites for reproduction and dispersal. If this effect is significant, then it will be of great interest as applied to the dispersal dynamics of *G. buenoi*, and the implications for its population biology (Spence, 1981, 1989).

5.2 Future directions

The *A. glabra-G. buenoi* system is fairly characteristic of eugregarine relationships, yet it is unique in some respects offering some unique opportunities for further work. In particular, the mode of transmission is interesting, as gerrids are the first semi-aquatic, hemipteran insects reported to harbour gregarines. Understanding the infection process is the next crucial step in grasping how this system functions. Furthermore, this would allow gerrids to be colonized within the laboratory, opening the doors to controlled experiments.

Gregarines are often characterised as being highly host specific, often to the level of species (Levine, 1988). However, growing evidence shows that gregarines often infect a suite of hosts, but are more successful and less pathogenic in one particular host,

referred to as the "target" or "primary" host (Clopton and Gold, 1996; Lien and Levine, 1980; Munsterman and Wesson, 1990). This combination, greater success and lower pathogenicity reflect one evolutionary strategy, i.e. for the parasite to evolve a benign relationship with its host. Classical theory suggests that parasites are more damaging to non-target hosts because they lack this co-evolved relationship; consequently, gregarines have been used in biological control programs focusing on their non-target hosts (Beier and Craig, 1985; Walsh and Olson, 1976).

The detailed impact of this gregarine on non-target hosts was not investigated in this study, and would complete the definition of this host-symbiont assemblage. *A. glabra* was able to complete development in both *G. comatus* and *G. pingreensis* but was far less prevalent.

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